Bipolar DBP Update 2009

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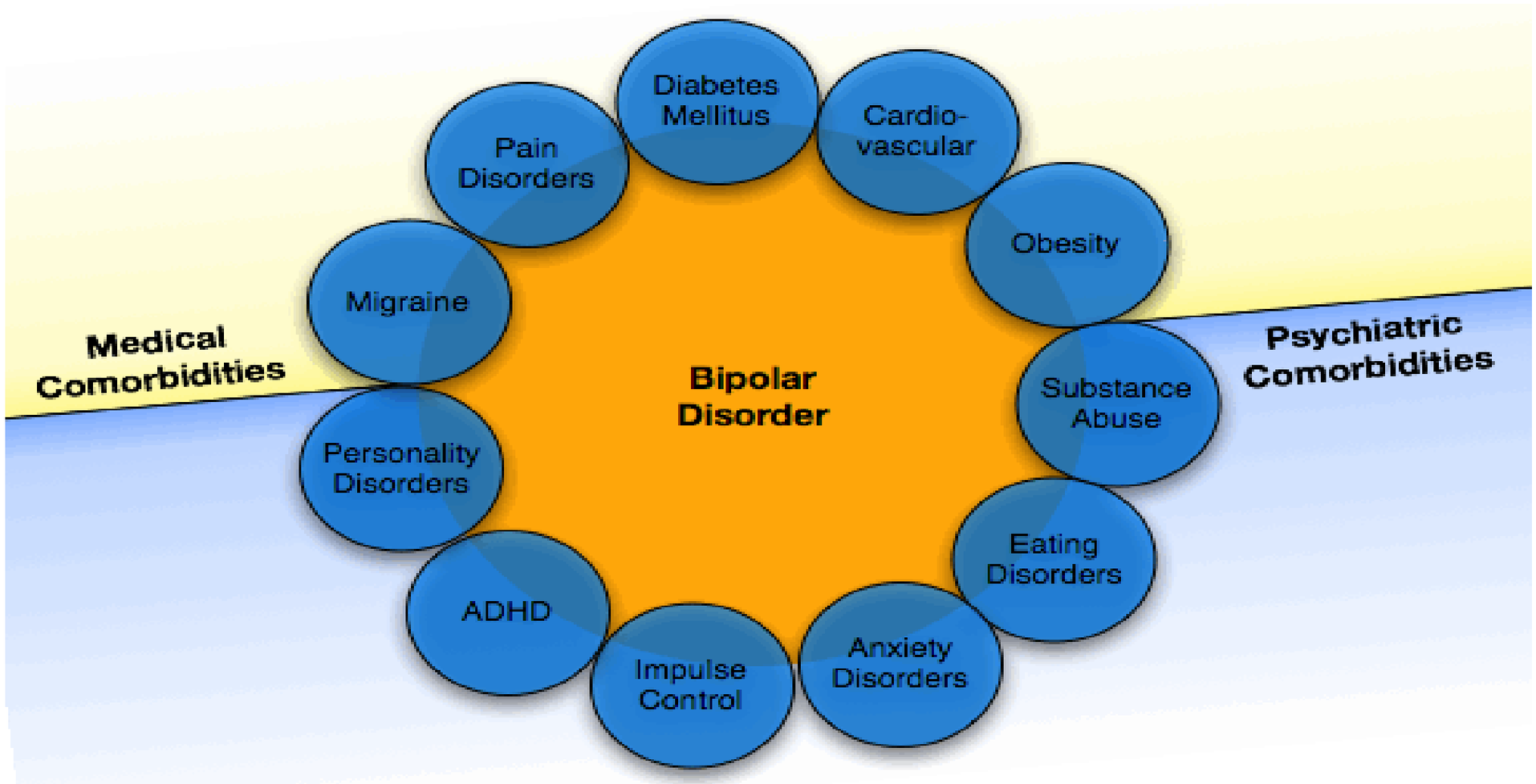
NCIBI Fourth Annual Research Meeting

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



McIntyre RS, et al. *Human Psychopharmacol.* 2004;19:369-386.

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

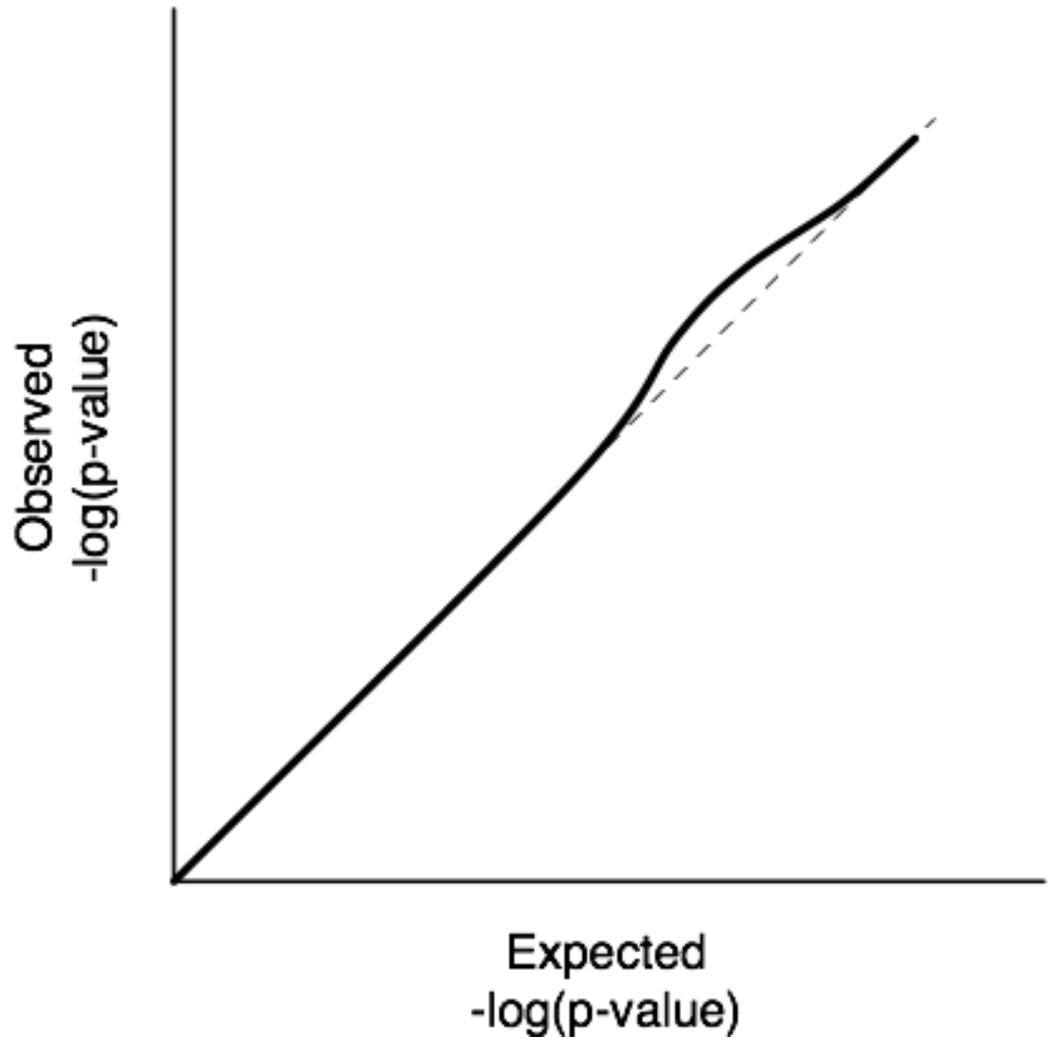
- Ferreira *et al*. Nature Genetics, 2008
  - *CACNA1C ($p< 10^{-8}$)* and *ANK3 ($p<10^{-9}$)*
- Scott *et al*. (Pritzker collaboration) PNAS, 2009
  - *MCTP1*, *NEK4* and *ITIH1 ($p<10^{-7}$)*
- Smith *et al*. (NIMH “G11” Collaboration) Mol. Psychiatry, 2009
  - GAIN - European and African American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

Association: Majority of “significance” at 10^{-4}

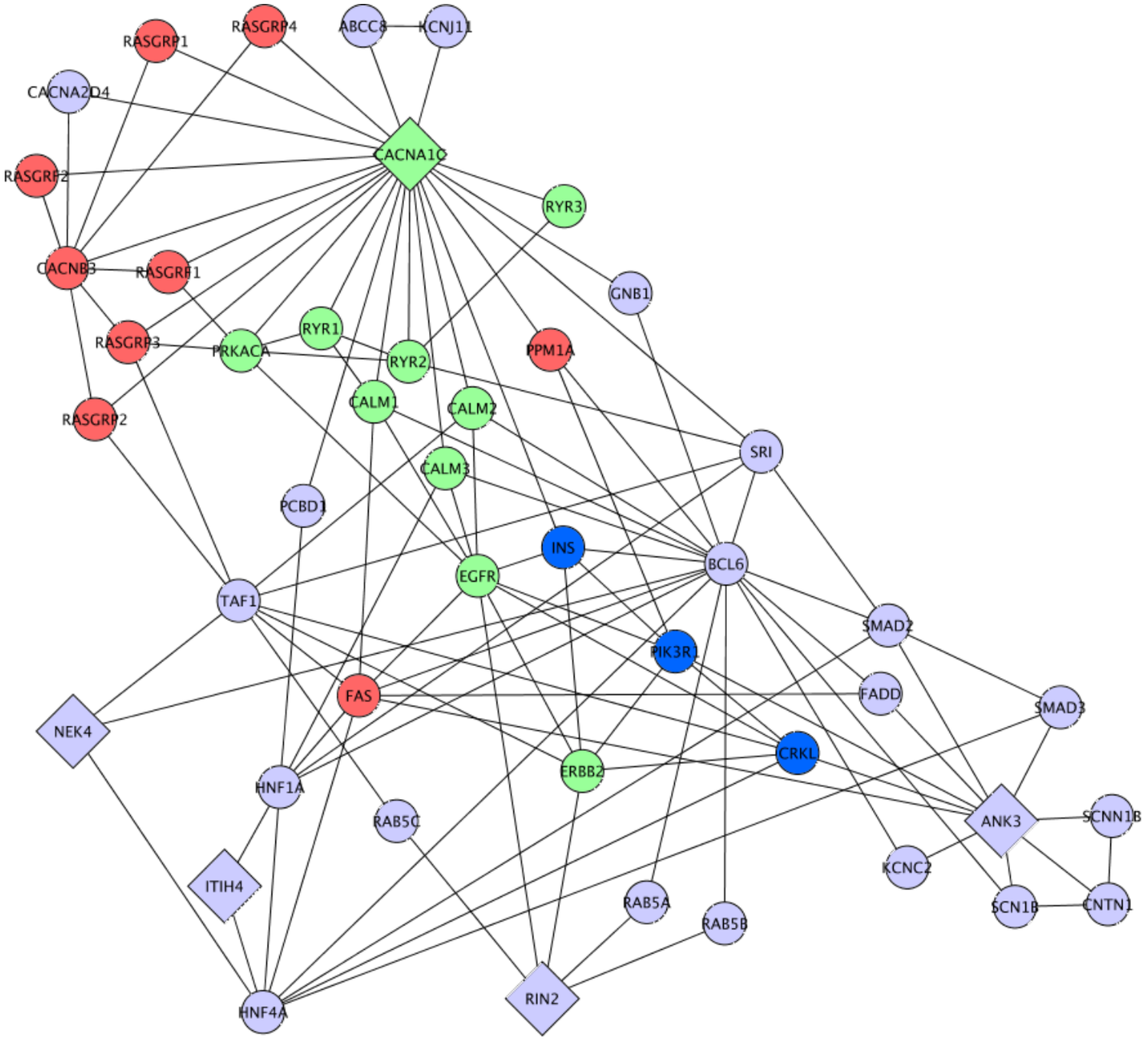
- GWAS of common disorders identifies a small number of markers with significant *p*-values that survive multiple-testing correction.

- 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

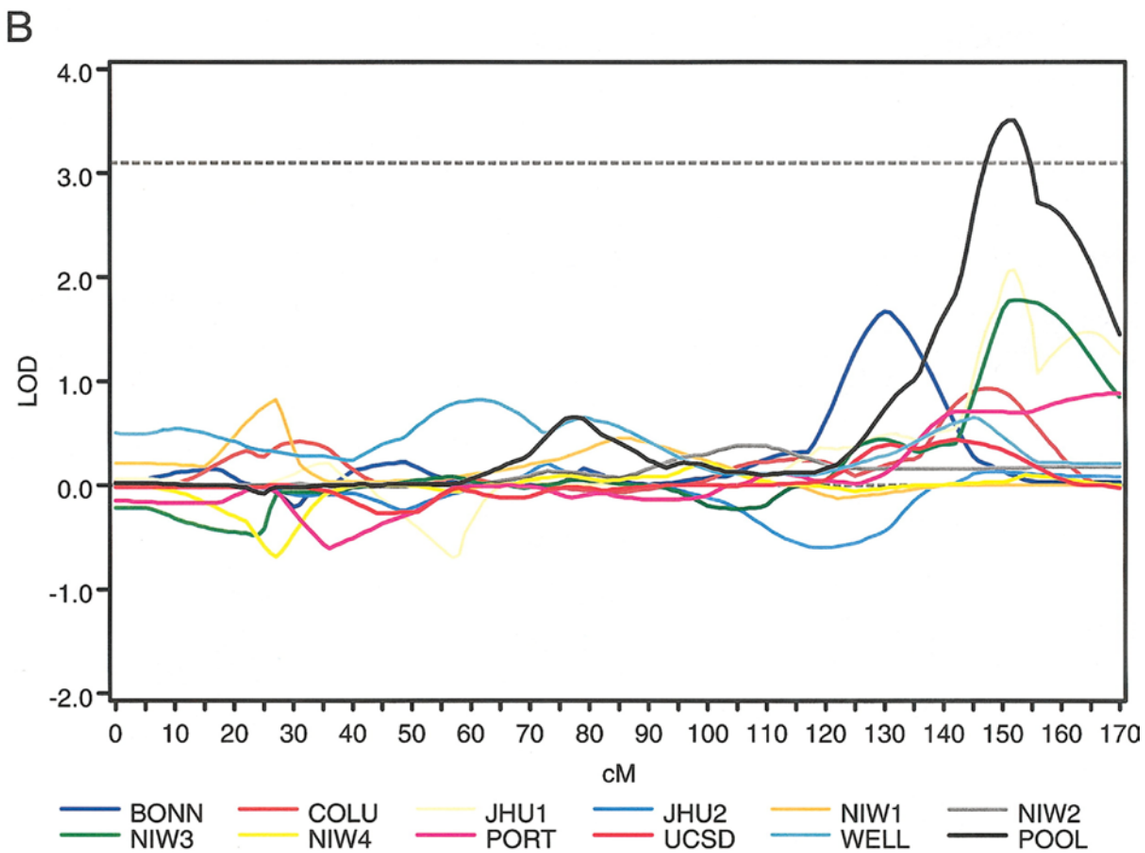
Rare Variants – Variable Copy Number

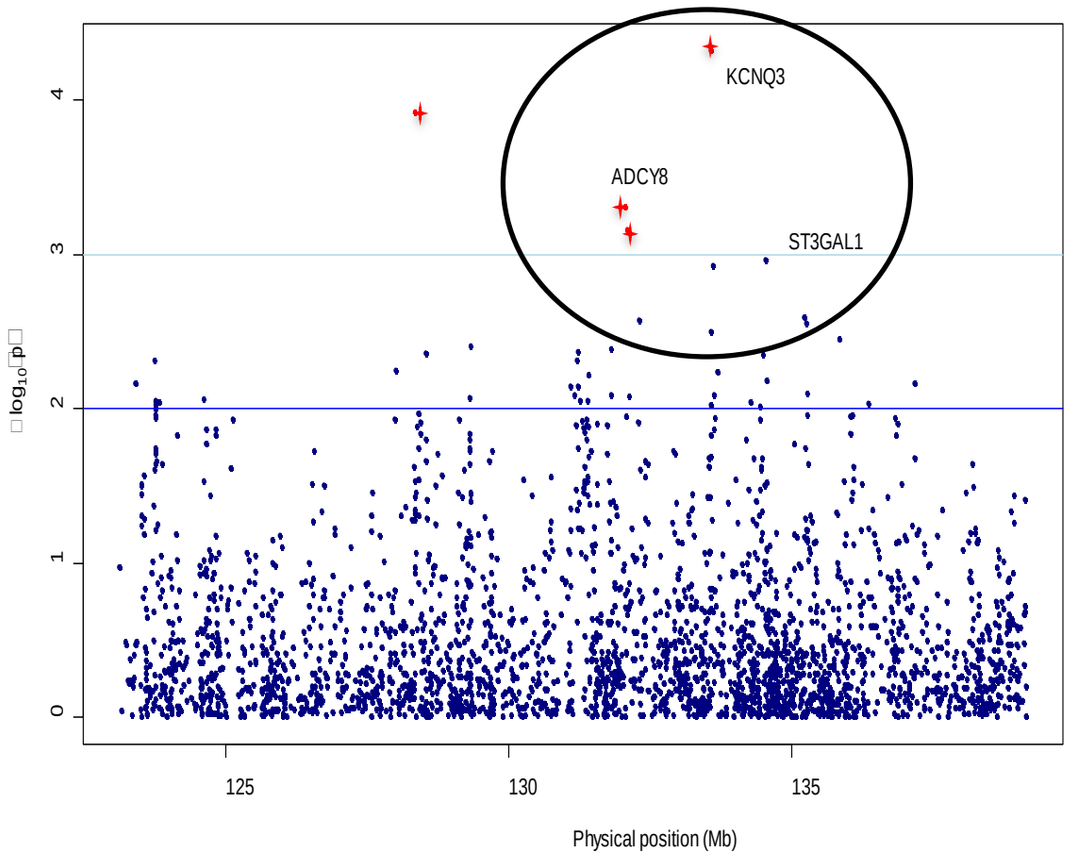
- Are Rare variants driving risk behind Bipolar Disorder?
  - 3 Publications in 2008 identified CNVs increased in schizophrenia
  - Birdsuite tools (Broad) applied to GAIN (NIMH) BP dataset (“G11” - Zhang et al, Mol Psychiatry 2009)
  - Identified evidence for singleton deletions increased in BP (16%) 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)





ADCY8 KCNQ3 ST3GAL1

Emerging Genes of Interest in BP Disorder

Recent Literature

- *CACNA1C*
- *ANK3*
- *DGKH*
- (*NEK4*)
- (*ITIH4*)
- (*RIN2*)

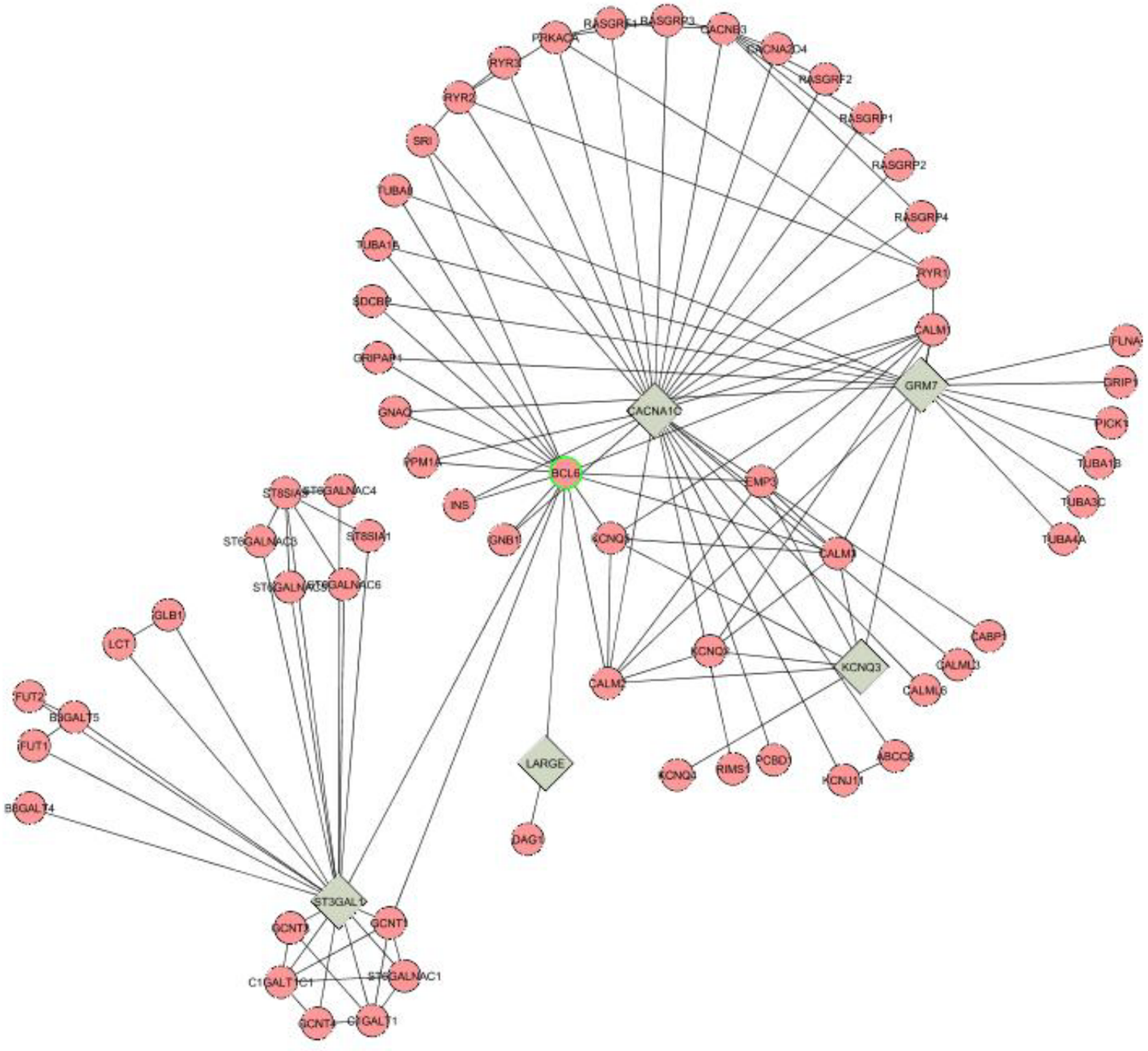
8q24 Findings

- *KCNQ3*
- *ADCY8*
- *ST3GAL1*

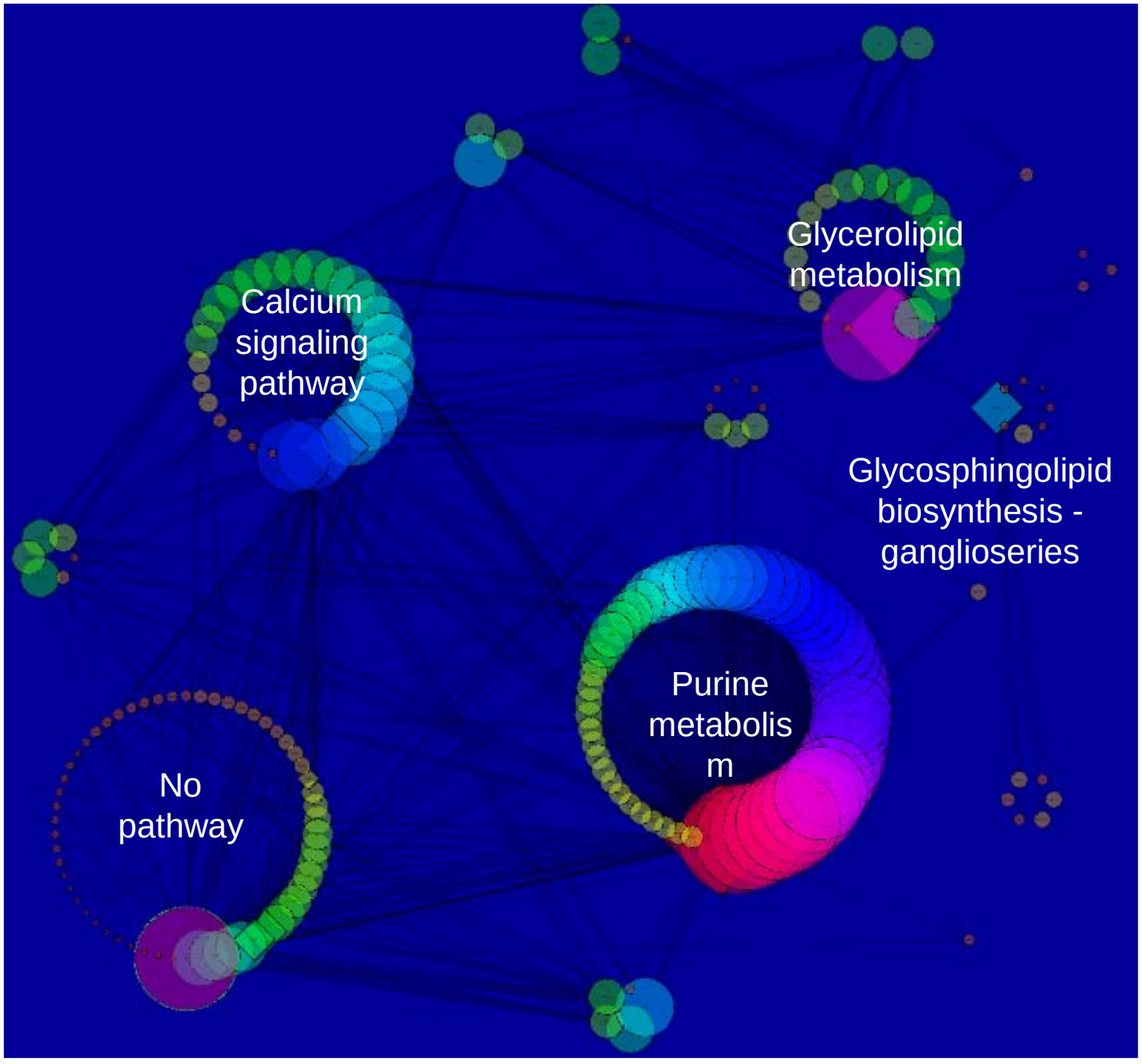
CNV report

- *GRM7*
- *LARGE*

Genetic interactions per MiMI



Calmodulin Gene Linkers (top KEGG Pathways)



CACNA1C

DGKH

ST3GAL1

LARGE

KCNQ3

ANK3

GRM7

ADCY8

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

0 mM lithium

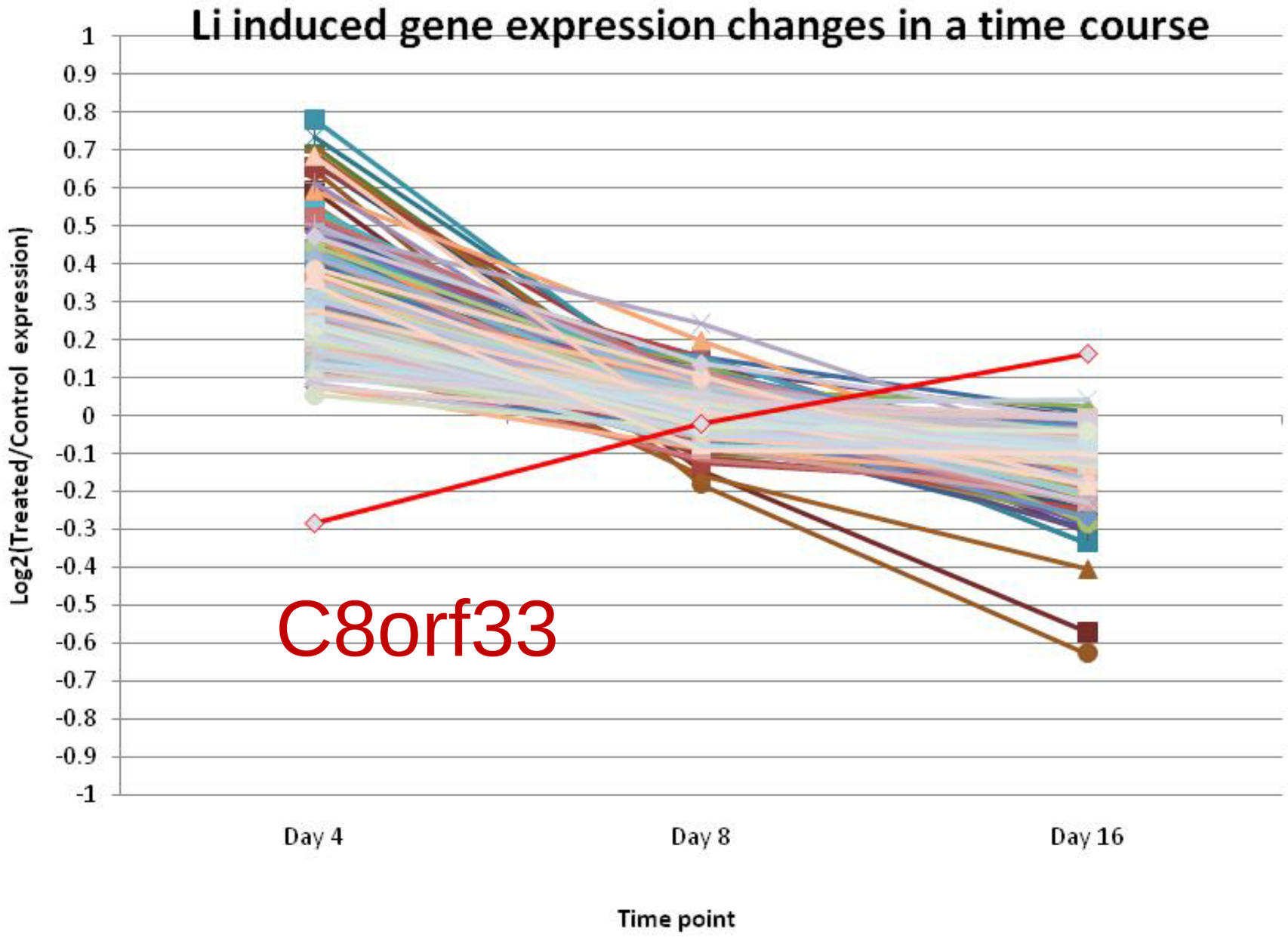
Sample 5 mL culture at day 4, 8, 16 for microarray analysis

Isolate total RNA for microarray expression assays

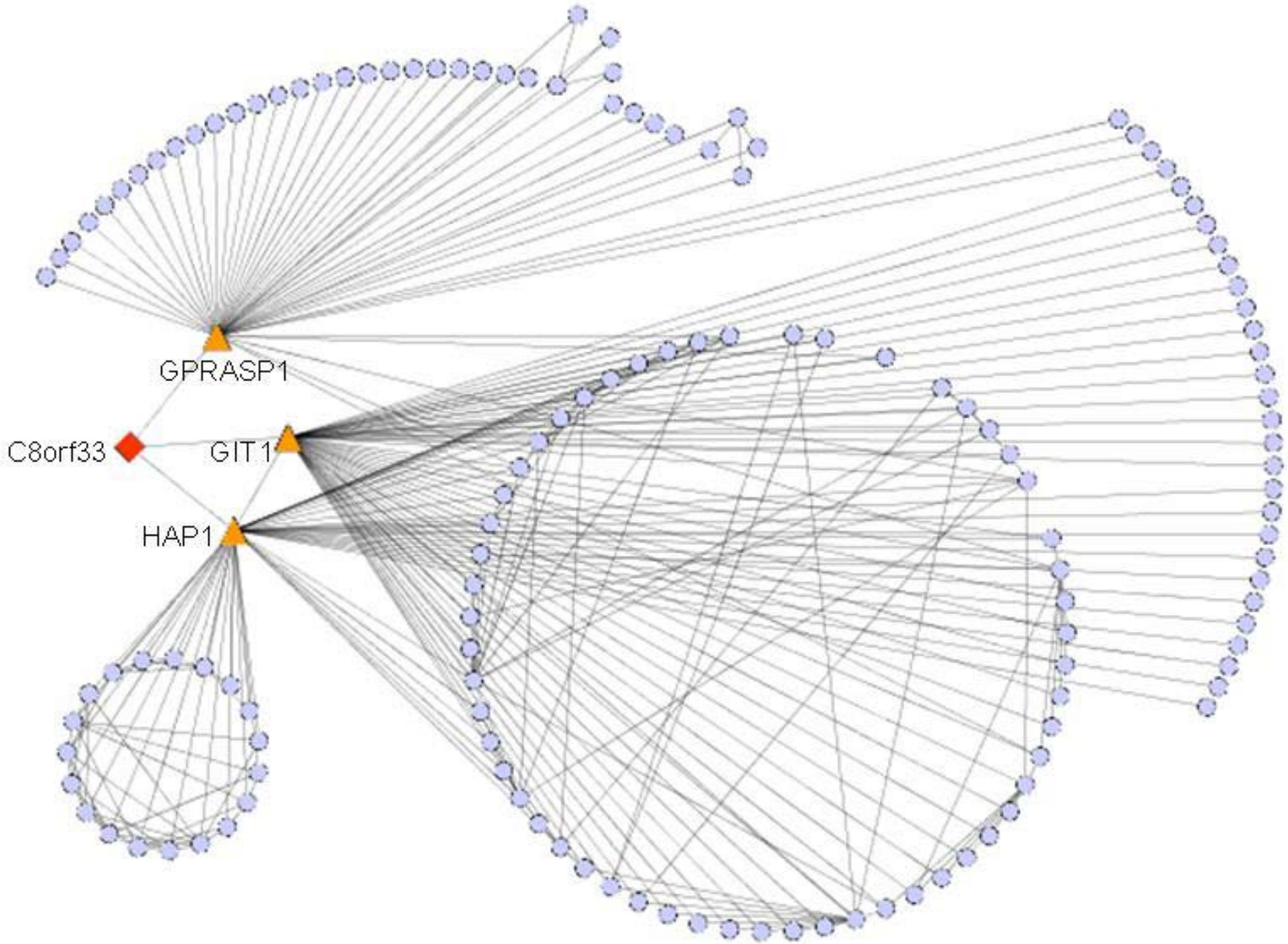
Lithium Mediated Expression – Key Messages

- 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%)



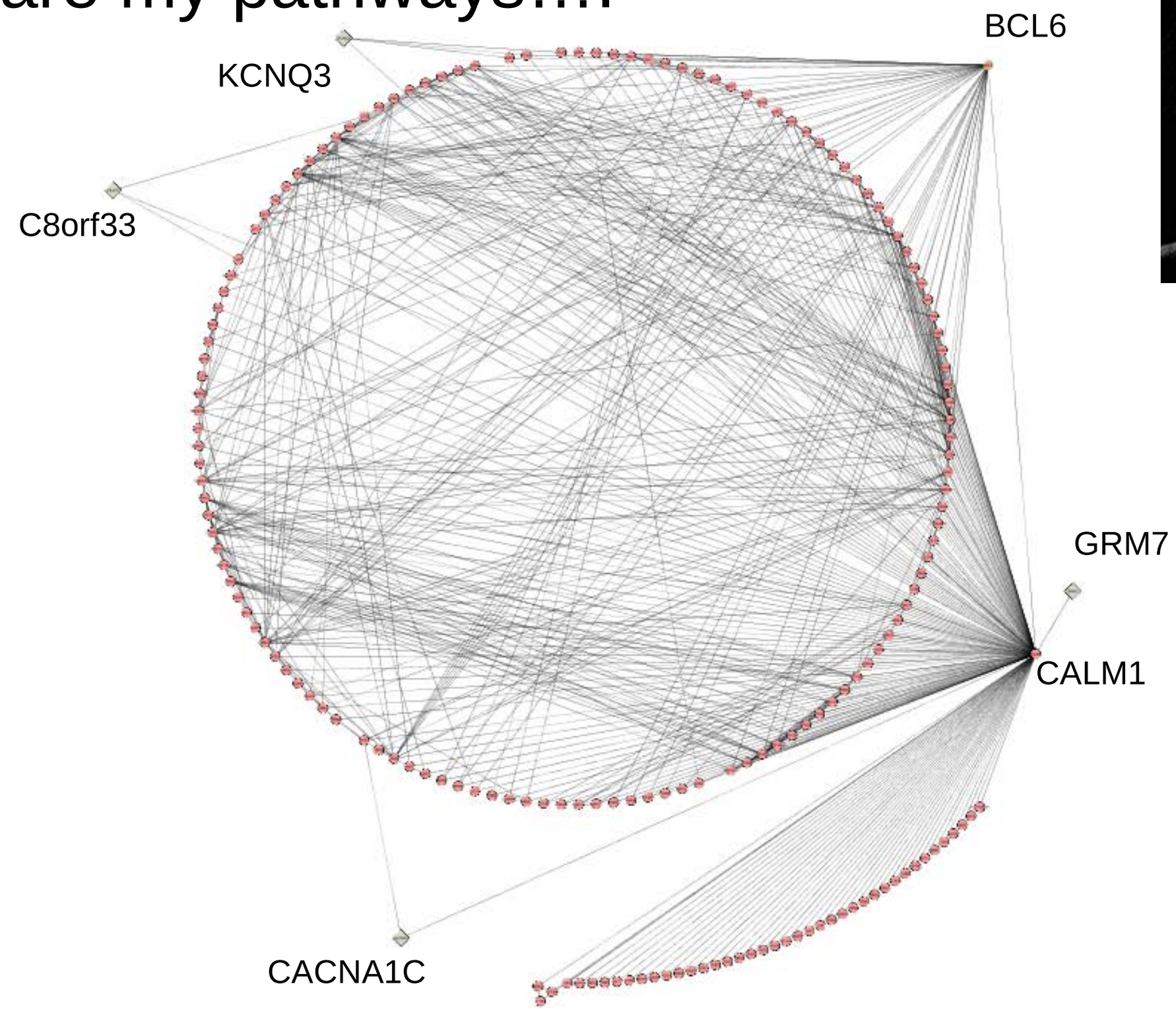
C8orf33 interacts through HAP1-GPRASP1-GIT1, and the extended gene interaction network



GO term enrichment of the 144 genes from the C8orf33 gene interaction network

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni_P
GO_TERM_BP	GO:0019932~second-messenger-mediated signaling	20	95	204	8550	4.85E-13	4.75E-10
GO_TERM_BP	GO:0007186~G-protein coupled receptor protein signalin	33	95	792	8550	2.32E-11	2.27E-08
GO_TERM_BP	GO:0007242~intracellular signaling cascade	35	95	945	8550	1.08E-10	1.05E-07
GO_TERM_BP	GO:0007187~G-protein signaling, coupled to cyclic nucle	14	95	108	8550	1.29E-10	1.26E-07
GO_TERM_BP	GO:0019935~cyclic-nucleotide-mediated signaling	14	95	111	8550	1.83E-10	1.79E-07
GO_TERM_BP	GO:0007166~cell surface receptor linked signal transduct	39	95	1296	8550	2.23E-09	2.18E-06
GO_TERM_BP	GO:0007165~signal transduction	55	95	2523	8550	1.36E-08	1.33E-05
GO_TERM_BP	GO:0007154~cell communication	57	95	2797	8550	6.99E-08	6.83E-05
GO_TERM_BP	GO:0007188~G-protein signaling, coupled to cAMP nucle	10	95	75	8550	1.10E-07	1.08E-04
GO_TERM_BP	GO:0019933~cAMP-mediated signaling	10	95	77	8550	1.39E-07	1.36E-04
GO_TERM_BP	GO:0007217~tachykinin signaling pathway	4	95	6	8550	2.51E-05	2.46E-02
GO_TERM_BP	GO:0007193~G-protein signaling, adenylate cyclase inhit	5	95	17	8550	2.92E-05	2.86E-02
GO_TERM_BP	GO:0007213~acetylcholine receptor signaling, muscarinic	4	95	7	8550	4.36E-05	4.27E-02
GO_TERM_MF	GO:0042277~peptide binding	17	96	131	8854	4.28E-13	4.19E-10
GO_TERM_MF	GO:0001653~peptide receptor activity	14	96	88	8854	6.69E-12	6.54E-09
GO_TERM_MF	GO:0004930~G-protein coupled receptor activity	27	96	629	8854	1.16E-09	1.13E-06
GO_TERM_MF	GO:0060089~molecular transducer activity	41	96	1651	8854	9.33E-08	9.13E-05
GO_TERM_MF	GO:0004871~signal transducer activity	41	96	1651	8854	9.33E-08	9.13E-05
GO_TERM_MF	GO:0008227~amine receptor activity	8	96	39	8854	1.52E-07	1.49E-04
GO_TERM_MF	GO:0001584~rhodopsin-like receptor activity	22	96	549	8854	2.42E-07	2.37E-04
GO_TERM_MF	GO:0004888~transmembrane receptor activity	29	96	977	8854	6.78E-07	6.63E-04
GO_TERM_MF	GO:0004872~receptor activity	33	96	1312	8854	3.04E-06	2.97E-03
GO_TERM_MF	GO:0042165~neurotransmitter binding	8	96	80	8854	2.20E-05	2.15E-02
GO_TERM_MF	GO:0004985~opioid receptor activity	4	96	6	8854	2.34E-05	2.29E-02
KEGG_Pathway	hsa04080:Neuroactive ligand-receptor interaction	26	57	222	3366	7.38E-16	7.22E-13
KEGG_Pathway	hsa04020:Calcium signaling pathway	18	57	171	3366	7.90E-10	7.73E-07
KEGG_Pathway	hsa04810:Regulation of actin cytoskeleton	13	57	183	3366	3.16E-05	3.09E-02

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

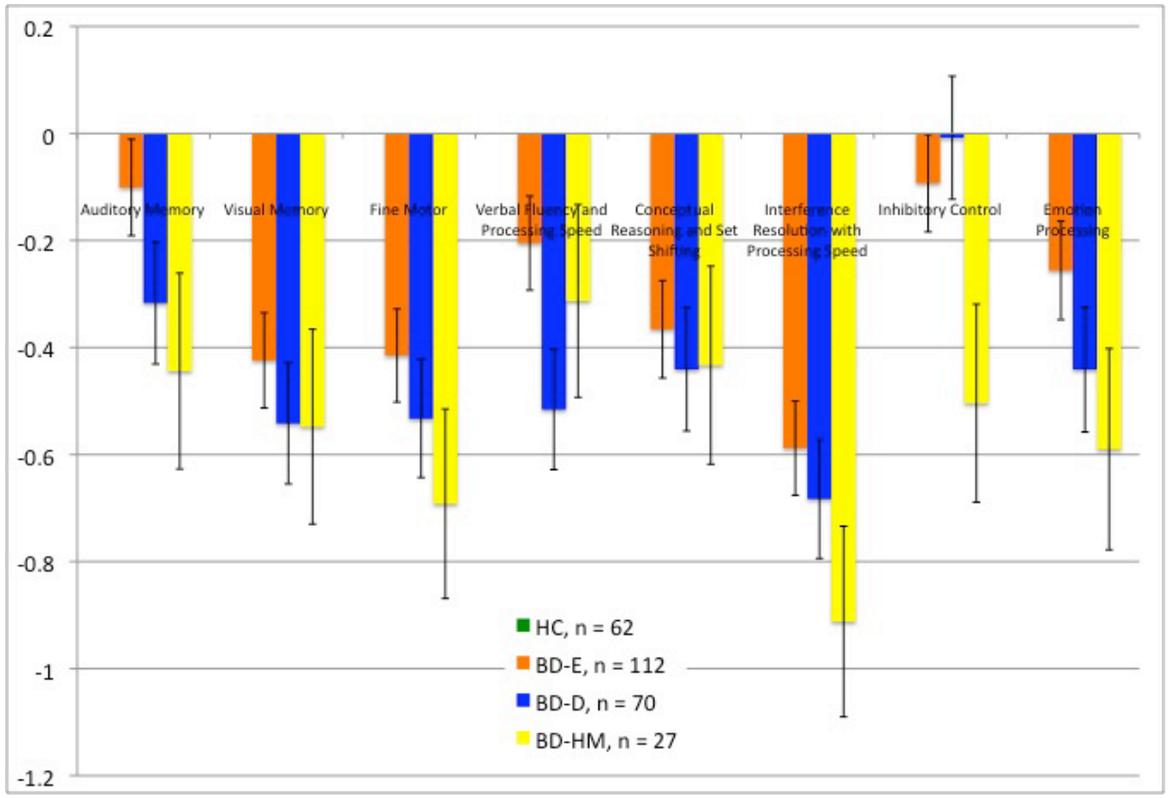
- Inositol Monophoshatase (*IMPA2*) was significantly decreased by 21% at day 4 – with no significant changes at day 8 or 16

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

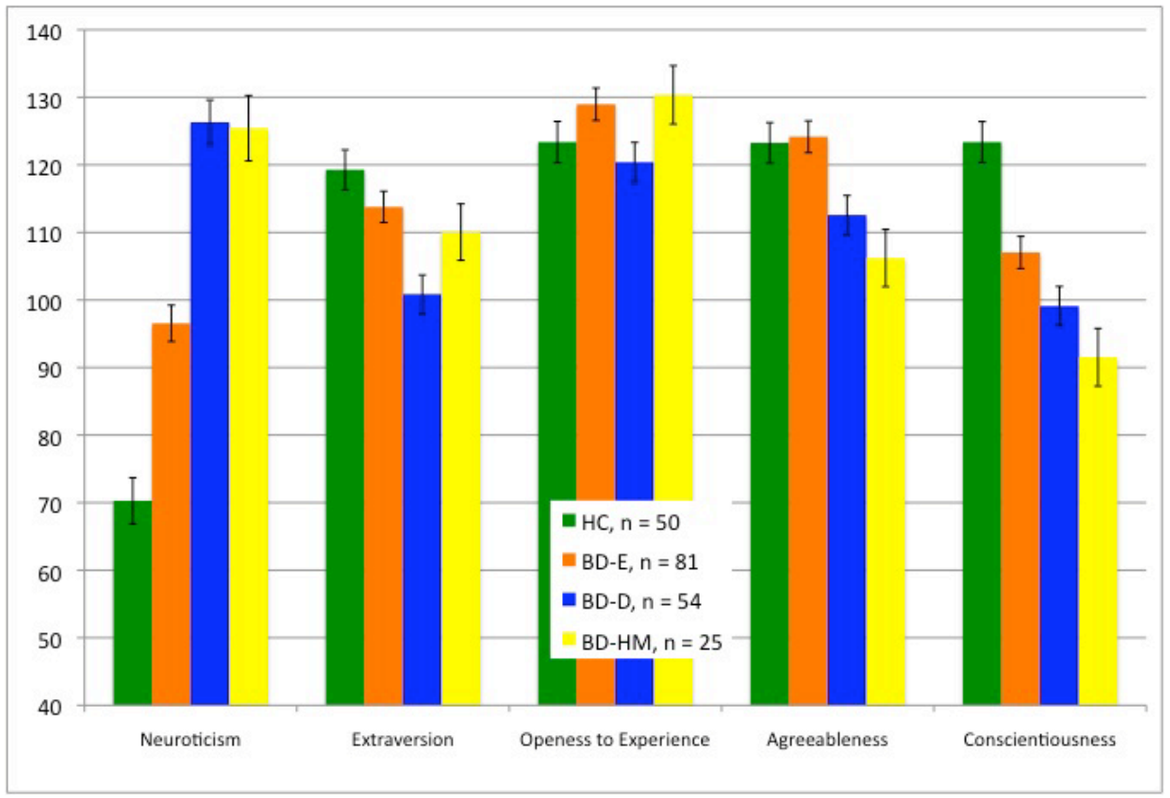
- Calmodulins showed no significant changes.

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.



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)

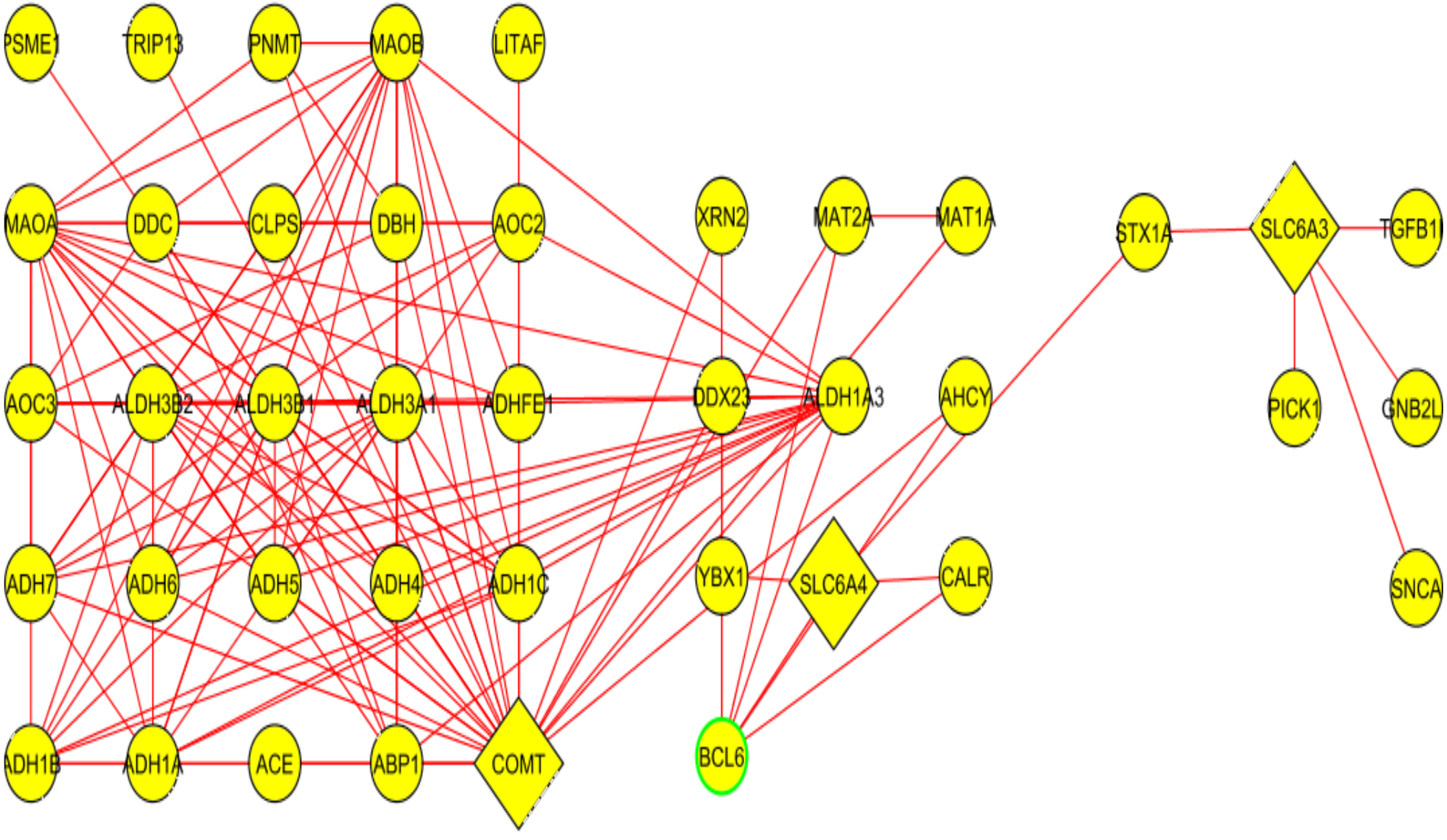
- **PDG-ACE** (Predicting Disease Genes from Analysis of Common Elements) finds significant commonality among these candidates

    - Psychiatric, substance use, attention deficit
    - Gender specific effects in both psychiatric and substance use disorders

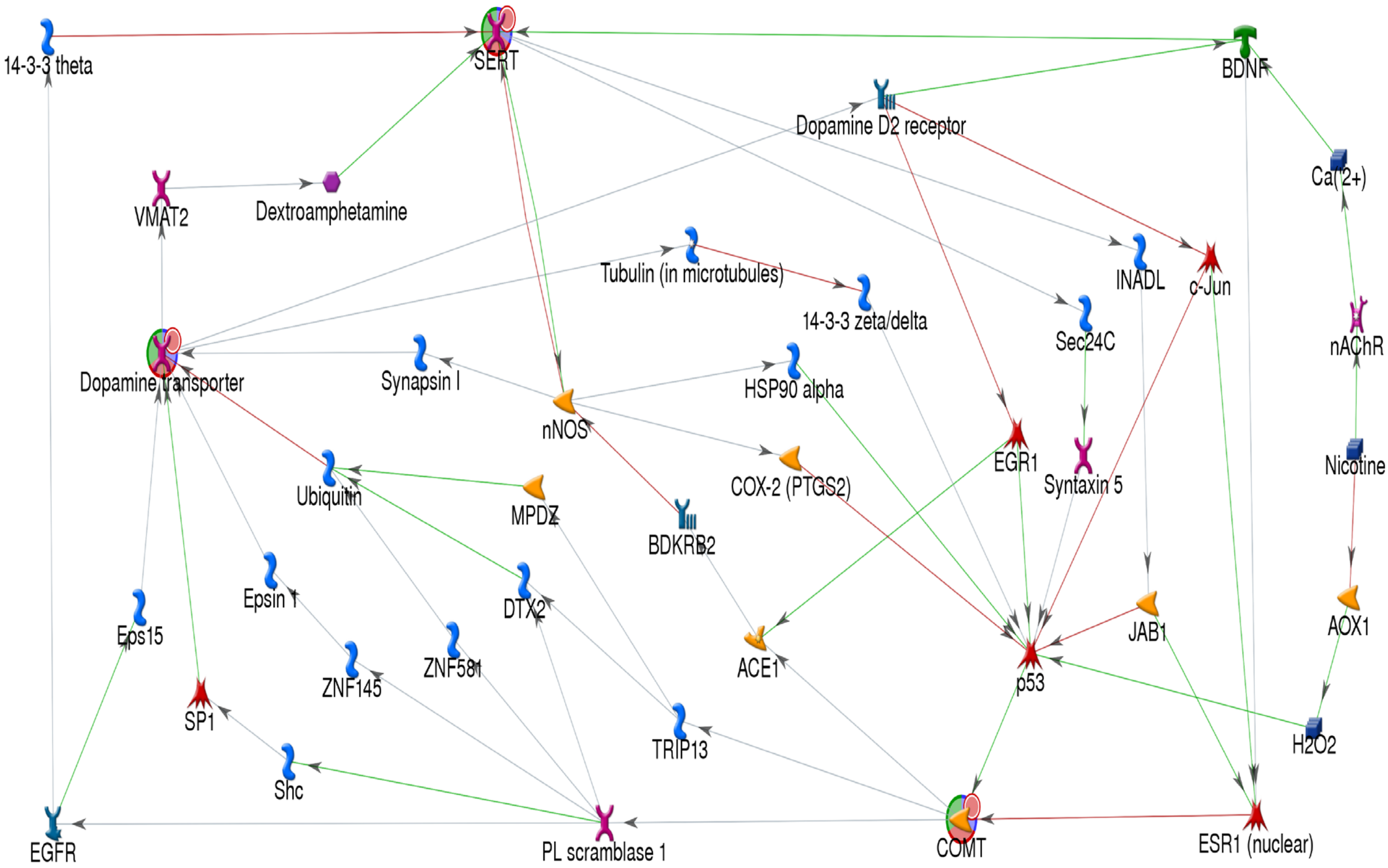
Building Bridges: Network Modeling

- 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, Neuro-physiology, Neuropsychology, Biochemistry, Metabolomics, and Immunology.