Lithium Induced Regulation of Genetic Transcription in Bipolar Lymphoblastoid Cell Lines

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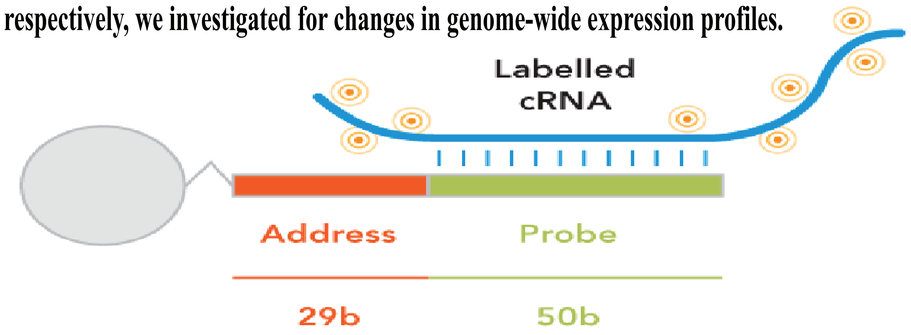
Introduction

Lithium (Li) is one of the most efficacious treatments for Bipolar Disorder (BD). Recent research on the genetics of BD has implicated genes affiliated with cell signaling and ion channels (CACNA1C, ANK3, and DGKH). Lithium is known to interact with and inhibit GSK3b and IMPases to modulate the wnt and phosphoinositol signaling pathways. However, much remains unknown with regards to the downstream gene expression changes affected by the regulation of these pathways.

Methods

We cultured Lymphoblastoid Cell Lines (LCL's) from the whole blood of 10 subjects diagnosed with BD. These cells were divided into 2 groups (one group bathed in Lithium at clinically relevant concentrations and the other group bathed in saline) and evaluated for gene expression changes over 16 days. On days 4, 8, and 16 respectively, we investigated for changes in genome-wide expression profiles.

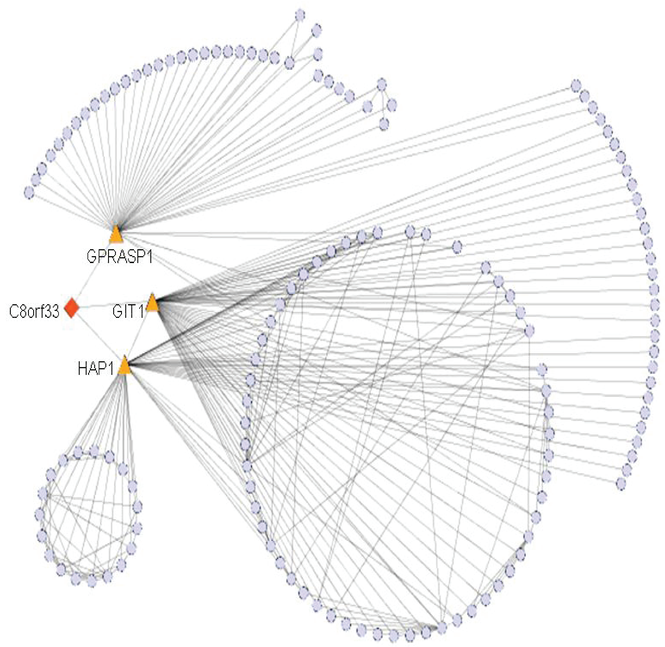


We utilised the Illumina Ref8_V2 platform which included 22184 probes to search for changes in genetic transcription. Image analysis and data normalization was accomplished with BeadStudio and Lumi software. SAM (Serial Analysis of Microarrays) was employed to identify significant genes and to estimate the FDR. MiMI (Michigan Molecular Interactions, NCIBI) was employed to identify the gene interaction networks. EASE (Expression Analysis Systematic Explorer) was employed for GO term enrichment analysis.

Results

Time point (TP) Analysis	#genes (FDR <5%)	Up	Down	Oposite Dir
Day4_TP	2789	1559	1230	-
Day8_TP	892	314	578	-
Day16_TP	1577	478	1099	-
Common in 3 time points	43	7	28	8
Two_classPaired_3TP (slope change)	218	1	217	-

MiMI identification of C8orf33 gene interaction network.
Shown are 144 genes (nodes) and 345 interactions (edges).



EASE functional analysis of the 144 genes (nodes) in the C8orf33 network.
Shown are the GO terms resulting from the EASE analysis.

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 signaling pathway	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 nucleotide second messenger	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 transduction	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 nucleotide second	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 inhibiting pathway	5	95	17	8550	2.92E-05	2.86E-02
GO_TERM_BP	GO:0007213~acetylcholine receptor signaling, muscarinic pathway	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

Conclusions

These data outline the lithium induction of hundreds of genes (FDR < 5%) from Bipolar lymphoblastoid cell lines.
This provides further evidence in support of a genetic hypothesis in the explanation of lithium's mechanism of action.
MiMI has been useful in identifying the gene interaction networks involved with those genes whose expression was induced by lithium exposure.