Lithium response in bipolar disorder correlates with improved cell viability of patient derived cell lines

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Supplementary figure 1. A) Characterization of LCLs by immunophenotyping- Scatterplot and histogram plot showing LCLs positive for CD19 (B cell marker) (top panel), negative for CD3 (T cell marker) (middle panel), negative for CD56 (NK cells) (bottom panel). B) Representative flow cytometer scatter plot for mitochondrial potential and cell viability assay. Dot plot for MTDR signal against Sytox Green, further gated into four quadrants based on live or dead cells (Sytox positive indicate dead cells) and high or low MMP population (based on MTDR signal intensity). The percentage of cells in Q1 (dead cell) and Q3 (live cells with high MMP) were analyzed further to assess cell viability and MMP respectively. C) Mitochondrial membrane depolarization positive control experiment- Histogram plot showing the change in mean MFI of MTDR after incubation with CCCP (50uM) and PFA (2%). D) Representative flow cytometer scatter plot for cell cycle assay. Dot plot for PI, to show gating of single cells using width versus area parameters. E) Illustrates PI area parameter histogram plot of the singlet cells to determine percentage of cells in G0/G1, S and G2/M phases of the cell cycle using FlowJo software. Abbreviations: LCLs, lymphoblastoid cell lines, PBMCs, peripheral blood mononuclear cells, EBV, Epstein-barr virus, MFI, mean fluorescence intensity, MTDR, mitotracker deep red, CCCP, carbonyl cyanide m-chlorophenyl hydrazine, PFA, paraformaldehyde.
### Supplementary table 1: Important studies related to mitochondrial function, cell death and cell proliferation in bipolar disorder

| Author and year | Sample | Methodology | Significant results |
|-----------------|--------|-------------|---------------------|
| **Studies related to mitochondrial function** | | | |
| Konradi et al., 2004 [1] | 9 BD and 10 control hippocampus from PM brains | Gene array to study mRNA expression in BD & control hippocampus | Expression of nuclear mRNA coding for mitochondrial proteins regulating oxidative phosphorylation in complexes I-V & ATP-dependent process were downregulated in hippocampus of BD. |
| Iwamoto, Kakiuchi, Bundo, Ikeda, & Kato, 2004 [2] | PM brain tissues:11 BD and 15 controls; LCLs: 14BD & 11 controls | mRNA levels analysis in BD PM brain tissues and LCLS | Altered mRNA levels of proteins involved in aberration of protein translocation system into mitochondria (affecting mitochondrial function) in LCLs and brain tissues of BD. |
| Andreazza, Shao, Wang, & Young, 2010 [3] | 15 each post-mortem DLFC from BD patients & control | Investigated ETC complex I activity & oxidative damage to mitochondrial proteins along with levels of complex I subunit NDUFS7 | Levels of NDUFS7 & complex I activity decreased significantly in BD. Protein oxidation & 3-nitrosine increased in BD compared to controls. |
| Regenold et al., 2012 [4] | PM brain cortex tissue from 15BD and controls | Studied Hexokinase1 (HK1) attachment to outer mitochondrial membrane (OMM) in BD brain tissue | Decreased HK1 attachment in BD compared to controls. HK1 attachment to OMM, a critical feature of brain energy metabolism and survival of neurons through prevention apoptosis. |
| Gubert et al., 2013 [5] | Plasma and PBMC samples from 12BD and 30HC. | Evaluated oxidative stress marker in plasma and ETC complex activities in PBMCs of BD | No significant difference in oxidative stress markers and ETC complex activities between BD and HC. |
| de Sousa et al., 2015 [6] | 24 HC and 25 BD; Patients treated with lithium for 6 weeks | Evaluated leukocyte ETC complex activities in BD & effect of lithium on ETC | No significant differences in mitochondrial ETC complex activities between BD and HC. Lithium treatment significantly increased mitochondrial complex I activities. |
| Yoshimi et al., 2016 [7] | CSF: 54 BD & 40 controls; PM brain tissue: 35BD & 34HC. | Evaluated the association of iso-citrate with BD | Iso-citrate levels significantly Increased in CSF from BD. mRNA levels of iso-citrate dehydrogenase in BD PM tissue was low compared to controls |
| Study                        | Participants                                                                 | Methodology                                                                 | Findings                                                                                                                                                                                                 |
|------------------------------|-------------------------------------------------------------------------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Scaini et al., 2017 [8]      | PBMC from 16BD and 16HC                                                       | Analysed levels of mRNA, protein and activity of mitochondrial related factors in PBMCs of BD and HC. | Levels of anti-apoptotic proteins and citrate synthase activity were significantly lower, while caspase activity was higher in PBMC of BD. Levels of mRNA, protein related to mitochondria fusion were lower & related to fission were higher in PBMC of BD. Showed mitochondrial dynamics & cell death pathway activation in BD, supporting link between mitochondria & pathophysiology of BD. |
| Bosetti et al., 2002 [9]     | Rat-Lithium treatment for 7 days (acute) or 42 days (chronic).                | Gene expression analysis of lithium treated rat brain                          | Chronic treatment at therapeutic concentration altered expression of several genes regulating mitochondrial enzymes in rat brain.                                                                         |
| Lai, Zhao, Warsh, & Li, 2006 [10] | Human SH-SY5Y neuroblastoma (1 or 7 days of treatment) | Looked into the effect of lithium (1mM) or valproate (0.6mM) in stress induced human neuroblastoma cells | Pretreatment of SH-SY5Y cells for 7 days with lithium or valproate significantly reduced rotenone or H2O2 induced cytochrome C release, caspase activity & cytotoxicity and upregulated BCL2 protein level. No effect was reported on 1 day treatment with the lithium or valproate. |
| Washizuka, Iwamoto, Kakiuchi, Bundo, & Kato, 2009 [11] | 1) LCLs from 25 BD1, 10 BDII & 33 HC. 2) 4 HC LCLs for lithium (0.75mM) or VPA (100ug/mL) treatment experiment | Studied gene expression of NDUFV2 in LCLCs of BD & HC; and after treatment of HC LCLS with lithium or VPA for 24hrs or 7 days | 1) NDUFV2 gene expression was significantly downregulated in BD1 and upregulated in BDII compared to HC. 2) VPA treatment significantly increased NDUFV2 compared to vehicle. |
| Maurer, Schippel, & Volz, 2009 [12] | Human PM brain cortex from 5 Controls treated with lithium (0.1mM-10mM) for 10min | Investigated the effect of lithium on respiratory chain enzyme activities after exposure to lithium in human brain tissue | ETC complexes were significantly increased dose dependently by lithium with max at 1mm. Succinate dehydrogenase was significantly increased at higher concentration of lithium |
| Study                          | Cell Line/Brain Tissue                                                                 | Methodology                                                                 | Findings                                                                                                                                                                                                                                                                                                                                 |
|-------------------------------|---------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Bachmann et al., 2009 [13]   | 1) SH-SY5Y cell line; 2) Brains from adult male Wistar Kyoto rats                    | Examined the effects of mood stabilizers on mitochondrial function and against mitochondrial mediated neurotoxicity | Cell respiration rate was enhanced by long term treatment with lithium or VPA. Mitochondrial function (membrane potential & oxidation) was enhanced by chronic lithium or VPA treatment in SH-SY5Y cells. In vivo: long-term treatment with lithium or VPA at therapeutic concentration prevented methamphetamine (meth) induced toxicity at the mitochondrial level (mitochondrial cytochrome c, anti-apoptotic Bcl-2/Bax ratio and COX activity). Oligo array analysis: pre-treatment with lithium or VPA prevented meth induced dysregulation of gene expression of proteins related to apoptotic pathway and mitochondrial functions. BCL2 expression increased after 6 days treatment. |
| Cataldo et al., 2010 [14]    | 1) PM prefrontal cortex: 10 BD & 10 controls; 2) Fibroblasts: 8 BD & 8 HC; 3) LCLs: 6 BD & 6 HC | Evaluated structure and distribution of mitochondria in BD compared to controls; Effect of therapeutic dosage of lithium in fibroblast after 5 days treatment | Ultra-structure examination revealed smaller mitochondrial areas in BD brain PFC. Altered mitochondria morphology & distribution was reported for BD fibroblasts and LCLs. Significant differences in cytochrome C distribution in BD fibroblasts. No significant differences in mitochondrial differences in either groups on treatment. However, significant difference for distribution of mitochondria between treated BD and treated HC. |
| Sitarz et al., 2014 [15]     | Fibroblasts from 5 POLG-deficient patients & 3 HC                                     | Effect of 10mM VPA for 10 days on mitochondria associated proteins          | VPA treatment increased mtDNA copy number. Protein levels of genes involved in mtDNA maintenance (POLG), mitochondria biogenesis & OXPHOS (COX2) increased significantly.                                                                                                                                                                                                                         |
| da Costa, Kormann, Galina, & Rehen, 2015 [16] | Neural progenitor cells from human embryonic stem cells | Effect of VPA (0.01,0.1, 1mM) for 24hours on cell size, mitochondrial morphology and function | Cell size and mitochondrial morphology changes after 1mM VPA treatment. Mitochondrial membrane potential (MMP) decreased with 1mM VPA.                                                                                                                                                                                                                             |
| Authors                              | Subjects                                                                 | Methods                                                                                       | Results                                                                                                                                         |
|--------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| Mertens et al., 2015 [17]            | 6BD and 4HC dentate gyrus neurons derived from fibroblasts               | Studied the mitochondria morphology and function in derived neurons from BD and HC.          | Increased MMP and mitochondrial gene expression in BD neurons. Size of neuronal mitochondria was smaller in BD compared to HC. Lithium treatment increased mitochondrial size in lithium responsive neurons, whereas MMP remained unaffected. |
| Kakiuchi et al., 2005 [18]           | 30 controls and 27 BD frontal cortex from PM brain tissue               | Examined mitochondrial DNA (mtDNA) copy number in BD                                        | No significant difference in mtDNA copy number of PM brain tissues between BD and controls.                                                      |
| Vawter et al., 2006 [19]             | PM brain tissues from 9BD & 20 controls                                  | Analysed mitochondrial related gene expression and mtDNA copy numbers in BD and controls     | 1) Mitochondrial gene & nDNA encoded mitochondrial genes were differentially expressed in BD. Mitochondrial related gene expression different in BD with lithium prescription Vs. BD without lithium at the time of death. The mtDNA copy number was non-significantly increased in BD compared to controls. |
| Sabunciyan et al., 2007 [20]         | PM frontal cortex from 40BD & 44 controls                                | Examined mtDNA copy number in BD & controls                                                 | No significant difference in mtDNA copy number of PM brain tissues between BD and controls.                                                      |
| Torrell et al., 2013 [21]            | PM brain tissues from 15BD & 15 controls                                  | Examined mtDNA copy number and MT-ND1 gene expression in BD & controls                       | MT-ND1 gene expression was significantly increased in BD compared to controls. No significant difference for mtDNA content in PM brain tissues between BD and controls. |
| C. C. Chang, Jou, Lin, & Liu, 2014)  [22] | Leukocyte from 40 BD & 70 HC                                             | Investigated mtDNA & oxidative damage in BD and HC leukocytes.                               | Leukocyte mtDNA copy number in BD was significantly lower than HC. Mitochondrial oxidative damage was significantly higher in BD compared to controls.     |
| de Sousa et al., 2014) [23]          | Leukocyte from 24 HC & 23 BD in depressive episode.                     | Evaluated mtDNA content in BD & HC. And tested if the content in BD varied after 6 weeks of lithium treatment. | No significant difference in mtDNA copy number between BD and HC at baseline. No difference was reported even after 6 weeks of lithium treatment in BD cases. |
| Gabriel R. Fries et al., 2017 [24]   | Peripheral blood samples from 22 BDI & 20 HC                            | Evaluated mtDNA copy number in BD & HC                                                      | The mtDNA copy number distribution in peripheral blood was significantly different in BD compared to HC. (Notably high variability in distribution was reported for BD group). |
| Study                                      | Type of Sample and Controls | Methodology                                                                 | Findings                                                                 |
|-------------------------------------------|----------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------|
| David Stacey et al., 2018 [25]            | Peripheral blood samples  | Comparison of mRNA expression levels between lithium responders and non-responders | 43 mRNA levels downregulated in lithium responders includes mitochondrial encoded genes- MT-ND1, MT-ATP6, MT-CyB. Genes involved in mitochondrial function pathway (ETC, OXPHOS) overexpressed. |
| Studies related to cell death              |                            |                                                                             |                                                                         |
| Shao & Vawter, 2008 [26]                  | PM DLPFC from 29 BD and 27 controls | Gene expression array analysis in brain tissues from BD & HC | Genes involved in nervous system development, cell growth, & cell death were dysregulated. |
| McCurdy et al., 2006 [27]                 | Olfactory mucosa from 8 BD & 10 HC | Examined the rate of cell death in BD compared to HC | Cell death was significantly more in BD compared to HC. |
| F M Benes, Matzilevich, Burke, & Walsh, 2006 [28] | PM brain tissues from 9 BD& 10 controls. | Gene expression array analysis in brain tissues from BD and controls | 19 of 44 genes related to apoptosis were upregulated. Antioxidant related genes were downregulated. |
| Herberth et al., 2011 [29]                | PBMCs from 16 BDI, 16BDII & 32HC; Validation in 7 BDI, 7 BDII & 14 HC. | Tested proteome profiling in PBMCs from BD & HCs. And effect of BD serum analytes on PBMCs from HC. | Proteome profiling of PBMC revealed differentially expressed proteins involved in cell death and survival pathways. Addition of BD serum analytes on PBMCs from HC subjects reduced cell survivality. |
| Kazuno et al., 2013 [30]                  | 1) LCLs from monozygotic twins discordant for BD. 2) 8 LCLs each from BD & HC to validate | Evaluated protein markers in whole cell lysate derived from LCLs of twins & validated. | Several proteins involved in cell death & glycolysis was significantly differentially expressed between the patient and the co-twin. Case- control analysis validated only upregulation of PGAM1 (involved in energy metabolism) in BD cases. |
| Gabriel Rodrigo Fries et al., 2014 [31]   | PBMC from 10 BD and 7 HC    | Assessed cell death & viability in PBMCs of BD & HC                           | Cells in early apoptosis was significantly higher in BD. No significant difference in cell viability, late apoptosis & necrosis between BD and HC. |
| Marianthi, Olga, Aristotelis, Nikolaos, & Fragiskos, 2015 [32] | Skin fibroblasts from 10 BD & 5 HC | Transcriptome profiling in fibroblasts from BD and HC | Genes involved in positive regulation of apoptotic process & cell cycle were differentially expressed in BD compared to HC. |
| Authors                     | Cells/Conditions                                                                 | Investigated                                                                 | Summary/Result                                                                 |
|-----------------------------|----------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Wollenhaupt-Aguiar et al., 2016 [33] | Neurons differentiated from neuroblastoma cell line (SH-SY5Y) challenged with serum of 12 BD or 6 HC | Investigated whether biochemical changes in the serum of patients induces neurotoxicity in neuronal cell cultures. | Reduced neurite density in neurons treated with serum of BD patients. Neurons challenged with serum of late stage patients showed significant decrease in cell viability. |
| Xiaohua Li, Bijur, & Jope, 2002 [34] | Review article                                                                  | Reviewed articles related to lithium or VPA treatment in human cell lines      | Higher concentration of lithium reduced GSK3B activity & blocked facilitation of apoptosis. VPA provided protection from apoptosis by inhibiting pro-apoptotic (such as reduced caspase 3 activity). |
| A. J. Kim, Shi, Austin, & Werstuck, 2005 [35] | Human hepatocarcinoma cell line (HEPG2)                                          | Studied the ER stress induced dysfunction after pre-treatment with 0.5mM VPA for 18hours | Pre-treatment with VPA increased the cellular resistance to ER stress induced dysfunction and protects from apoptosis by inhibiting GSK3B. |
| Lai et al., 2006 [10]        | Human SH-SY5Y neuroblastoma (1 or 7 days of treatment)                           | Looked into the effect of lithium (1mM) or valproate (0.6mM) in stress induced human neuroblastoma cells | Pretreatment of SH-SY5Y cells for 7 days with lithium or valproate significantly reduced rotenone or H2O2 induced cytochrome C release, caspase activity & cytotoxicity and upregulated BCL2 protein level. No effect was reported on 1 day treatment with the lithium or valproate. |
| Wilot et al., 2007 [36]      | Hippocampal slices of rats                                                       | Evaluated neuroprotective effect of lithium & VPA against ATP induced cell death in rat hippocampus | ATP induced cell death was significantly reduced by lithium or VPA treatment at therapeutic dosage in both in vitro (acute) and in vivo (chronic) experiments. |
| Go et al., 2011 [37]         | Neuronal progenitor cells (NPC) from embryonic brain of rats                     | Examined regulation of apoptotic cell death in rat NPCs by VPA                | VPA (0.2, 0.5mM) treatment decreased NPC cell death after growth factor withdrawal or H2O2 stimulated peroxide conditions. VPA upregulated BCL-XL protein & mRNA levels in concentration dependent manner and suppressed Bax levels. The result was confirmed by in vivo in developing rat brains. |
| Lowthert et al., 2012 [38] | 8 weeks of open label lithium study in 20 BD patients | To assess the change in gene expression in peripheral blood of lithium responders and non-responders | 127 genes differentially expressed between responders and non-responders. Pathway analysis showed regulation of apoptosis was significantly affected. Upregulation of anti-apoptotic gene BCL2 and downregulation of pro-apoptotic genes (BAD, BAK1) in responders and inverse relation in non-responders after 4 weeks. |
| Gawlik-Kotelnicka, Mielicki, Rabe-Jabłońska, Lazarek, & Strzelecki, 2016 [39] | Human neuroblastoma cell line (SH-SY5Y) | Assessed the effect of lithium (0.5 & 0.7mmol/L) for 24 hours in neuroblastoma cells. | Cell viability was significantly higher in therapeutic treated lithium samples than vehicle. |
| Del Grosso et al., 2016 [40] | Human oligodendrocyte cell line | Effect of lithium pre-treatment on cell viability | Psychosine induced autophagy in cells was rescued on lithium pre-treatment, by increasing cell viability. |
| Z. Li et al., 2017 [41] | Human neuroblastoma cell line (SH-SY5Y) | Effect of VPA in ER stress induced neuroblastoma cell lines on exposure to thapsigargin (TG) and on neuroprotection. | VPA treatment improves cell viability and reduces cell apoptosis in cell exposed to TG. ER stress induced apoptosis response protein were inhibited by VPA treatment. VPA upregulated the ratio of BCL2/Bax proteins in SH-SY5Y cells. VPA promotes cell proliferation through PI3K, AKT, GSK3B pathways. |
| Breen et al., 2016 [42] | LCLs from 23 Caucasian individual (8 BD lithium responders, 8 BD lithium nonresponders, 7 HC) | Exploring the effect of lithium 1mM (7 days) on transcriptome levels in LCLS from BD lithium response patients | Differential gene expression in apoptosis signalling system, defence response, protein processing pathways and response to ER stress pathways were discovered on treatment with lithium. |

**Studies related to cell proliferation**

| McCurdy et al., 2006 [27] | Biopsies of olfactory mucosa from 8 BD & 10 HC | Explored the cell proliferation rates in BD compared to HC | No significant differences in mitosis between the BD and HC, however 11 genes involved in cell proliferation & 4 in neurogenesis were differentially expressed in BD. Cell death was significantly more in BD compared to HC. |
| Author(s) | Samples | Methods | Findings |
|-----------|---------|---------|----------|
| F. M. Benes et al., 2007 [43] | Hippocampus tissues from PM brain of 7 BD & 7 controls | Gene expression profiling of brain tissues from BD and controls | GAD67 (glutamate decarboxylase 67) gene expression was significantly decreased in BD than controls and CCND2 (Cyclin D2) is known to regulate GAD67. CCND2 was significantly downregulated in CA2/3 region of brain tissues in BD. |
| Francine M Benes, Lim, & Subburaju, 2009 [44] | Hippocampus tissues from PM brain of 7 BD & 7 controls | Evaluate the expression profiling of genes involved in G1 & G2 checkpoints of BD | Genes associated with transcriptional complex & G1 or G2 checkpoint of cell cycle regulation in BD was differentially expressed. Gene included CCND2, CDK9 for G1 check point & P53, CHK2, CCNE for G2 checkpoint. |
| Marianthi et al., 2015 [32] | Skin fibroblasts from 10 BD & 5 HC | Transcriptome profiling in fibroblasts from BD and HC | Genes involved in positive regulation of apoptotic process & mitotic cell cycle were differentially expressed in BD compared to HC. |
| K. H. Kim et al., 2015 [45] | NPCs and matured neurons from 8 BD & 4 unaffected siblings | Transcriptomic microarray profiling in NPCs and matured neurons (early & late neurons) | Genes related to cell cycle were differentially expressed in BD late neurons compared to unaffected individuals. |
| Breen et al., 2016 [42] | LCLs from 23 Caucasian individual (8 BD lithium responders, 8 BD lithium nonresponders, 7 HC) | Exploring the effect of lithium 1mM (7 days) on transcriptome levels in LCLS from BD lithium response patients | Gene markers related to cell cycle and nucleotide excision repair were found to be differential in response to lithium between BD lithium responders and non-responders. |
| Mao, Hoang, & Dicorleto, 2001 [46] | Bovine aortic endothelial cells | Investigated the effect of lithium (5 & 10 mM) on regulation of cell cycle in bovine cells. | Lithium treatment increased G2/M cells without affecting cell viability up to 3 days whereas reduced thereafter. Lithium increased mRNA and protein levels of p21, cyclin dependent kinase inhibitors. Cyclin D mRNA expression was biphasic on lithium treatment--at 4 & 8 hours: It upregulated whereas at 24 & 48 hours: It was downregulated. |
| Authors                  | Study Population                                      | Methodology                                                                 | Findings                                                                 |
|-------------------------|-------------------------------------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Sun et al., 2007 [47]   | Human prostate cancer cells                           | Studied the effect of lithium in cell proliferation of prostate cancer cells. | Lithium (10mM) significantly inhibited cell proliferation at 72 hours treatment. Lithium significantly increased the percentage of cells in S phase & decreased cellular DNA replication. Lithium altered expression of gene regulating DNA replication & Cell cycle. Cyclin A, Cyclin E, P2I was downregulated whereas Cyclin D was upregulated. Protein level of Cyclin D was also up. |
| Seelan, Khalyfa, Lakshmanan, Casanova, & Parthasarathy, 2008 [48] | Human neuronal cell line (SK-N-AS) | Microarray expression profiling in human neuronal cell line after lithium (1.5mM) treatment for 33 days | Gene related to neuronal survival, growth, apoptosis, cell cycle regulation were differentially expressed on treatment with lithium. |
| Zanni et al., 2015 [49] | NPCs from mice                                         | Studied the effect of lithium (1mM or 3mM) in mice NPCs                      | Lithium attenuated the effect of irradiation exposure induced cell cycle arrest in G1 and G2 phase. |
| Rattanawarawipa, Pavasant, Osathanon, & Sukarawan, 2016 [50] | Stem cells from human exfoliated deciduous teeth      | Evaluated effect of lithium on cell proliferation in the cells after 3 days and 7 days treatment | Lithium significantly reduced colony forming unit ability/ proliferation in dose dependent manner. Lithium increased percentage of cells in subG0 phase, whereas decreased the percentage of cells in G1 phase after 3 days & 7 days of treatment. |
| Laeng et al., 2004 [51] | Rat neural stem cells                                  | RNA profiling and protein analysis of rat neural stem cell after VPA or lithium treatment | Cell cycle regulating genes – CCND2 was 5-6 fold increased on treatment by VPA, confirmed by protein analysis. Lithium treatment for 3 days increased CCND2 levels. |
| Catalano et al., 2005 [52] | Human papillary thyroid carcinoma cell line          | Tested the effect of VPA on cell cycle phases of human carcinoma cell line. | VPA increased subG1 population in time and dose (0.5-3mM) dependent manner. Growth arrest in G1 phase was increased by VPA (1 &3mM) treatment. Gene expression of P2I and Cyclin A was increased. G2/M cell population was decreased non-significantly by VPA treatment. |
| X. N. Li et al., 2005 [53] | Human medulloblastoma and                             | Investigated the effect of VPA (1 & 2.7mmol/L) on cell cycle phases of cell lines | VPA treatment caused cell cycle arrest for medulloblastoma cell line on day 7, i.e., significantly increased percentage of cells in G0/G1 phase & decreased cells in G2/M phase. |
| Study | Cell Type | Treatment | Findings |
|-------|-----------|-----------|----------|
| Wu & Guo, 2008 [54] | Immortalized human endometrial stromal cells | Examined the effect of VPA on cell cycle phases of stromal cell lines | VPA (3mM) for 16 hours treatment increased percentage of cells in G0/G1 phase and decreased cells in S phase & G2/M phases. |
| Witt et al., 2013 [55] | Primary murine prostate cancer cells (PCA) and fibroblasts | Studied the effect of VPA on CCND2 expression in murine cell lines | VPA treatment highly increased CCND2 gene expression in PCA cell line, however no effect of VPA on CCND2 expression was seen in murine fibroblast. |
| Claudia Morich, 2016 [56] | Tumour cell lines | Expression profiling after VPA treatment | CCND2 gene expression was significantly increased after VPA treatment. |
| Pietruczuk, Lisowska, Grabowski, Landowski, & Witkowski, 2018 [57] | T cells from 18 BD & 10 HC | Evaluated proliferation capacity & susceptibility to apoptosis in T cells and effect of lithium or valproate on these parameters | Cell cycle longer in BD compared to HC; reduced proliferation in lithium treated BD patients compare to HC and BD treated with VPA. Cell cycle longer in patients treated with VPA compared to lithium treated patients. In vitro exposure to VPA reduced cell division and cell proliferation irrespective of the disease state; lithium has no effect on proliferating capacity of T cells from BD patients. Higher doses of lithium shortened cell cycle. Apoptosis higher in BD cells, lithium and VPA prevents apoptosis in T cells from BD. BCl2 level: No significant difference between BD and HC |

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Supplementary table 2. Clinical and demographic characteristics of LCL study

| Variables                        | Total (N=25) | Responders (N=16) | Non-responders (N=9) | Chi square or z value | p value |
|----------------------------------|--------------|-------------------|----------------------|-----------------------|---------|
| Age at assessment                | 40.2(12.8)   | 37.6(10.5)        | 44.8(15.6)           | -1.0*                 | 0.3     |
| Gender-M: F (M %)                | 15:10(60%)   | 8:8(50%)          | 7:2(77.8%)           | 1.9*                  | 0.16    |
| Duration of illness (months)     | 239.2(146.9) | 214.8(93.3)       | 282.7(212.3)         | -0.19*                | 0.8     |
| Duration of lithium treatment (months) | 104.8(83.6) | 114.9(91.8) | 81.8(60.5) | -0.8* | 0.4 |
| Age of onset                     | 20.4(6.0)    | 20.4(7.3)         | 20.4(3.5)            | -0.8*                 | 0.3     |
| Number of hospitalizations       | 3.7(4.8)     | 3.8(5.8)          | 3.5(2.2)             | -0.9*                 | 0.3     |
| Psychotic symptomsa              | 20(83.3%)    | 13(81.3%)         | 7(87.5%)             | 0.15b                 | 0.69    |
| Total no. of episodes            | 8.4(6.2)     | 7.6(5.0)          | 10.1(8.4)            | -0.7*                 | 0.4     |
| No. of manic episodes            | 6.7(5.4)     | 5.8(4.3)          | 8.5(7.5)             | -0.7*                 | 0.5     |
| No. of depression episodes       | 1.8(2.5)     | 1.5(2.2)          | 2.5(3.2)             | -0.7*                 | 0.4     |
| No of mixed episodes             | 0.3(0.6)     | 0.2(0.5)          | 0.4(0.7)             | -0.5*                 | 0.5     |
| Family H/o BD                    | 15(62.5%)    | 8(50%)            | 7(87.5%)             | 3.5b                  | 0.06    |
| Family H/o psychosis             | 3(13%)       | 1(6.7%)           | 2(25%)               | N/A                   | N/A     |
| Suicide attempta                 | 2(9.1%)      | 2(13.3%)          | 0                    | N/A                   | N/A     |
| Onset episode                    |              |                   |                      |                       |         |
| Mania- 19 (76%)                  | 11 (71.4%)   | 8 (85.7%)         | 0.56b                | 0.45                  |
| Depression-6 (24%)               | 5 (28.6%)    | 1 (14.3%)         | 0.56b                | 0.45                  |
| ALDA total score                 | 5.6(3.0)     | 7.5(0.6)          | 2.2(2.4)             | -4.1*                 | 0.000*  |
| A score                          | 7.88(3.1)    | 9.8(0.5)          | 4.4(2.9)             | -4.4*                 | 0.000*  |
| B score                          | 2.7(1.2)     | 2.3(0.7)          | 3.5(1.4)             | -2.1*                 | 0.03*   |

*a Lifetime History, *p<0.05 (statistically significant); Values are mean (±SD), or n (%). a Mann-Whitney U test or b Pearson chi square were utilized to calculate p values across the variables between the responders and non-responders.
Supplementary table 3: Rare damaging exome variants identified in family A

| Gene   | Variant   | RS id/Novel | No of affected | Presence in BD1/BD2 | Signaling pathway                          | Cellular role                                                                 |
|--------|-----------|-------------|----------------|----------------------|--------------------------------------------|------------------------------------------------------------------------------|
| DENND5A | c.A2699G  | rs779817963 | 4              | BD1/BD2              | ERK pathway [1]                            | Cell migration [2], proliferation, apoptosis                                  |
| KIF7   | c.G2690C  | rs749711306 | 3              | BD1                  | Hedgehog signalling [3,4]                   | Cell proliferation [5], migration                                             |
| SCN3A  | c.G83A    | rs775711350 | 3              | BD1/BD2              | No pathway reported                        | Neuronal migration [6], Cell cycle [7]                                        |
| PARP14 | c.G3467A  | Novel       | 4              | BD1/BD2              | JNK2 signalling [8]                         | Apoptosis [9], glycolysis [10]                                                |
| PCCB   | c.C595T   | rs371155999 | 3              | BD1/BD2              | Propionlate metabolism pathway [11]        | Mitochondrial oxidative phosphorylation [12]                                 |
| TRMT44 | c.C1405T  | rs373816157 | 3              | BD1                  | MAPK and ERBB pathway [13,14]              | Cell migration, proliferation [15,16], oxidative stress [17]                 |
| NRG2   | c.C1477T  | rs148371256 | 3              | BD1                  | MAPK and ERBB pathway [13,14]              | Cell migration, proliferation [15,16], oxidative stress [17]                 |
| NIPBL  | c.A4496C  | Novel       | 4              | BD1/BD2              | Notch pathway [18], Cohesion [19], Wnt and PI3K-AKT pathway [18] | Cell migration, proliferation, apoptosis [20,21]                            |
| SCUBE3 | c.C1996T  | Novel       | 3              | BD1/BD2              | FGF Pathway [22] and hedgehog signaling [23] | Cell proliferation [24]                                                      |
| ANLN   | c.C128T   | rs575071809 | 3              | BD1/BD2              | PI3K pathway [25]                          | Cell migration, Cell cycle [26,27]                                            |
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### Supplementary table 4: Studies of BCL2, GSK3B and NR1D1 genes in bipolar disorder

| Author and Year         | Type of study                                    | Sample details                          | Objective of study                                      | Significant results                                                                                                                                                                                                 |
|-------------------------|--------------------------------------------------|-----------------------------------------|---------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| H.-W. Kim, Rapoport, & Rao, 2010 [1] | Case- control study (protein/mRNA level)       | 10BD &10 control frontal cortex from PM brains | To investigate levels of BCL2 protein & mRNA in BD compared to HC | Decreased BCL2 protein and mRNA levels in frontal cortex from BD brain tissues compared to controls. BAX/BCL2 ratio was increased in BD brain tissues.                                                                 |
| Moutsatsou et al., 2014 [2] | Case- control study (mRNA level & apoptotic activity) | Lymphocyte from 35 BD and 10 HC       | To investigate the level of BAX/BCL2 mRNA ratio level, caspase3 activity and cytochrome C release in BD and controls. | Higher BAX/BCL2 mRNA levels in BD patients in manic and depressed state compared to HCs. Cytochrome c release, caspase-3 activity was increased in manic and depressed BD patients compared to HC, indicating higher apoptotic activity in BD. |
| W. T. Chen, Huang, & Tsai, 2015 [3] | Case- control study (protein level)             | 20 BD patients in manic phase and 40 HC from Taiwan | To examine the serum BCL2 levels in BD and HCs | Serum BCL2 levels higher in manic state of BD patients than HC, though statistically not significant.                                                                                                               |
| Uemura et al., 2011 [4] | Functional study and case- control association study | LCLs from 245 patients (150 BD-I, 65 BD-II &30 MDD) and 70 HC subjects | To study the role of BCL2 rs956572 SNP on basal intra cellular calcium, mRNA and protein levels in BD and control subjects. | 1) No significant association of the BCL2 SNP with any of the disorders. 2) Basal calcium levels of LCLs: Significantly higher in BD compared to controls, subjects with GG genotype being the highest compared to other genotype (AA<AG<GG). BD with GG reported to have higher levels compared to other groups carrying same GG. 3) BCL2 mRNA and protein levels were lower in BD than HC. GG genotype subjects lower levels compared to AA or AG, effect was more prominent in BD. |
| Soeiro-de-Souza et al., 2013 [5] | Case-control association study | 40 BD euthymic patients and 40 HC from Brazil. MRS is done to obtain glutamate levels of anterior cingulate cortex (ACC) | Tested the association of \textit{BCL2} (rs956572) SNP with ACC glutamate levels | AA genotype at the \textit{BCL2} SNP was associated with elevated ACC glutamate metabolites in the BD patients, not in controls. |
|--------------------------------|---------------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Uemura, Green, & Warsh, 2015 [6] | Functional study and case-control association study | LCLs derived from 215 BD cases, including 150 BD-I and 65 BD-II; and 70 healthy controls | To investigate whether the \textit{BCL2} rs956572 variant associates with intracellular calcium dyshomeostasis in BD and HC | 1) Lower Ca$^{2+}$ in subjects with BCL2 rs956572 AA variant compared to AG or GG as a whole. 2) BD patients carrying GG genotype - highest Ca$^{2+}$ compared to AA or AG. 3) BD patients carrying GG genotype - higher Ca$^{2+}$ compared to HC with GG genotype. |
| Corson, Woo, Li, & Warsh, 2004 [7] | Pharmacological study | Human hNT neurons from NT2 teratoma cells and SVG p12 SV40 glia cells (treated with lithium or valproate for 7 days) | To test the effect of lithium and valproate on \textit{BCL2} mRNA levels in hNT neurons | Treatment of hNT cells with valproate (0.35, 0.75, 1 mM) for 7 days upregulated \textit{BCL2} mRNA (max increase with 0.75mM), whereas lithium (0.75-2mM) could not alter. The SVG glia cells \textit{BCL2} mRNA was not changed by both the treatments. |
| Lai et al., 2006 [8] | Pharmacological study | Human SH-SY5Y neuroblastoma, SVGp12 glial cells and U87 glioma cells (treated with lithium [1mM] or valproate [0.6mM] for 1 or 7 days) | Looked into the effect of lithium or valproate in stress induced human cells | Pretreatment of SH-SY5Y cells for 7 days with lithium or valproate significantly reduced rotenone or H$_2$O$_2$ induced cytochrome C release, caspase activity & cytotoxicity and upregulated \textit{BCL2} protein level. No effect was reported on 1 day treatment with the lithium or valproate. Other cell types were not affected by any such treatments. |
| Creson, Yuan, Manji, & Chen, 2009 [9] | Pharmacological study | Human SH-SY5Y neuroblastoma cells | Looked in the effect of valproate on \textit{BCL2} protein and mRNA levels in human neuroblastoma cells. | Increased BCL2 protein in concentration (0.125-2mM) & time (1-3 days on 0.8mM) dependent manner. 0.5-2mM for concentration and 2 or 3 days treatment induced the increase in BCL2 level whereas 1 day did not. Similarly, valproate increased the BCL2 mRNA in time dependent manner, 3$^{rd}$ day being the highest increase. The enhancement of BCL2 levels was selective as the HK gene GAPDH was not altered. |
| Authors                          | Type                  | Duration/Description                                                                 | Study/Experiment                                                                                     | Results/Outcomes                                                                                           |
|---------------------------------|-----------------------|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| GSK3B                            |                       |                                                                                      |                                                                                                      |                                                                                                            |
| Odeya D, Galila A, & Lilah T, 2018 [10] | Pharmacological study | 10-12 weeks old wild type mice from IMPA1 colony                                    | Effect of lithium on BCL2 gene expression in hippocampi of mice                                      | On treatment with lithium BCL2 gene expression levels were increase when normalized to ACTB, whereas decreased on normalizing with MAPK6 or ANKRDI1. |
| Machado-Vieira et al., 2011 [11] | Functional study      | LCLs from 18 BD subjects with equal numbers of individuals carrying the rs956572 variants for AA, AG & GG | To study the role of BCL2 rs956572 SNP on basal intra cellular calcium and after 1mmol/L lithium treatment in BD LCLs for 7 days. | 1) Basal and stimulated intracellular Ca2+ were higher in BD LCLs with AA variant compared to GG variant though, BCL2 mRNA and protein level was least in AA variant of the BCL2 polymorphism. 2) Li treatment increased the BCL2 expression in the AA variants of BD LCLs. |
| Lowthert et al., 2012 [12]      | Pharmacogenetic study | 8 weeks of open label lithium study in 20 BD patients                                 | To assess the change in gene expression in peripheral blood of lithium responders and non-responders | 127 genes differentially expressed between responders and non-responders. Pathway analysis showed regulation of apoptosis was significantly affected. Upregulation of anti-apoptotic gene BCL2 & downregulation of pro-apoptotic genes (BAD, BAK1) in responders and inverse relation in NR after 4 weeks. |
| Xiaohong Li, Liu, Cai, Wang, & Li, 2010 [13] | Case-control study and anti-manic treatment response (protein level) | 1) 30 medication free BD manic subjects were compared with 30 healthy controls from Beijing Anding Hospital, China. 2) 47 BD (4 weeks) and 28 BD (8 weeks) subjects were analyzed for pre and post treatment with lithium, valproate and atypical antipsychotics. | To test the regulation of GSK3B in BD patients with manic episode and in response to treatment - examined the protein level and the inhibitory serine phosphorylation of GSK3B in PBMCs of patients compared with healthy controls | 1) The total protein levels of GSK3B was significantly higher in BD manic subjects than in healthy controls. 2) Phospho-Ser9-GSK3B was reported to be trend toward lower in bipolar manic subjects than in healthy controls. 3) Significant increase in phospho-Ser9-GSK3B was reported in 28 BD subjects post 4 weeks and 8 weeks treatment. 4) Total GSK3B among the 47 subjects was not significantly different at treatment. |
| Study Authors | Study Type | Sample Size | Methodology | Results |
|---------------|------------|-------------|-------------|---------|
| Pandey, Ren, Rizavi, & Dwivedi, 2010 [14] | Case-control study (protein level) | 1) 21 BD patients and 21 HC from Chicago were investigated for GSK3B level in platelet, 2) The patients compared for GSK3B level before and after treatment (lithium or valproate or antipsychotics) for 8 weeks. | To explore the role of GSK3B in BD and in response to treatment | 1) GSK3B protein level in cytosol and membrane fraction of platelets from BD were decreased in comparison to controls. 2) The protein level after 8 weeks of treatment was increased. |
| Lesort, Greendorfer, Johnson, 1999 [15] | Case-control study in PM brain tissues | DLPFC of 5 BD and 5 controls from Ohio. | To compare the levels of GSK3B protein in brain tissues of BD and HCs | No significant difference was reported. |
| Munkholm, Peijs, Vinberg, & Kessing, 2015 [16] | Case-control candidate gene expression study | 37 rapid cycling BD patients and 40 HC of Danish population | Tested GSK3B gene expression in BD & HC | GSK3B mRNA level was significantly downregulated in BD, however after Bonferroni correction the result was not significant. |
| Benedetti, Bernasconi, et al., 2004 [17] | Candidate gene association study | 185 Italian BD patients | To test the effect of the GSK3B rs334558 SNP on age at onset of BD | Homozygote TT was reported to be associated with earlier age at onset of BD. |
| Benedetti, Serretti, et al., 2004 [18] | Candidate gene association study | 60 depressed BD patients | To test the effect of GSK3B -50T/C polymorphism on age at onset of BD and acute response to total sleep deprivation | Homozygotes for the mutant allele was associated with later age at onset of BD, less severe symptomatology when depressed (HDRS score), & better acute effects of total sleep deprivation treatment on perceived mood (VAS score). |
| Nishiguchi, Breen, Russ, St Clair, & Collier, 2006 [19] | Case-control candidate gene association study | 280 Caucasian BD patients and 407 HC. | To test the association of the GSK3B -50T/C SNP and BD. | No significant association |
| Study Authors & Year | Study Design | Study Population | Study Aim | Findings |
|----------------------|--------------|------------------|-----------|----------|
| Szczepankiewicz, Skibinska, et al., 2006 [20] | Case-control candidate gene association study | 416 Polish patients and 408 HC | To test the association of the GSK3B -50T/C SNP and BD. | 1) Trend association of heterozygous T/C genotype with BD was reported. 2) Significant association of SNP with the female BDII patients (n=57). 3) No significant association with AAO of BD |
| Serretti et al., 2008 [21] | Candidate gene association study | 365 Italian mood disorder patients, included 122 MDD and 243 BD patients. | To evaluate the association of the polymorphism with symptomatic and personality feature in mood disorder | The GSK3B polymorphism was found associated with delusional symptomatology and with the personality features linked to self-transcendence. |
| Subhashree et al., 2009 [22] | Case-control association study (NIMHANS) | 186 subjects with BD and 186 healthy controls from NIMHANS, Bangalore, INDIA | To investigate the association of -50T/C SNP in GSK3B gene with BD. | No significant association |
| E. Jiménez et al., 2013 [23] | Candidate gene association study | 192 Caucasian BD subjects included 66 suicide attempters and 126 suicide non attempters | To investigate association between the GSK3B -50T/C SNP & suicide behavior in BD | C allele of the GSK3B SNP showed trend association (p=0.052) with suicide attempters. |
| Esther Jiménez et al., 2014 [24] | Candidate gene association study | 199 Caucasian BD subjects | To evaluate the effect of the SNP on impulsivity in BD | C allele carrier was associated with higher level of impulsivity in BD |
| Tang et al., 2013 [25] | Meta-analysis study | 48 relevant studies screened and 5 BD studies (3 Asian and 2 Caucasian) finally included for analysis. Total 971 cases and 1397 controls | To test the association of the GSK3B -50T/C SNP and BD. | No significant association of the GSK3B -50T/C SNP and BD. |
| G. Chen et al., 2014 [26] | Meta-analysis study | 95 relevant studies screened and 10 BD studies included 1) For association with BD | To investigate the association between the GSK3B -50T/C SNP and the susceptibility or age at | No significant association of the GSK3B -50T/C SNP with risk of BD or age at onset of BD was reported in any of the genetic model that was analyzed. |
| Author(s)                          | Study Type            | Details                                                                 | Findings                                                                 | Notes                                                                 |
|-----------------------------------|-----------------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------|
| De Sarno, Li, & Jope, 2002 [27]    | Pharmacological study | 1) **In vitro**: Human neuroblastoma SH-SY5Y cells- with valproate or lithium for 24 hours at varying concentration  
2) **In vivo**: Adult male C57BL/6 mice treated with 0.4% lithium (0.7mM at serum) | Evaluated the effect of lithium or valproate on phosphorylation of GSK3B. | 1) Sodium valproate treatment effected gradual increase in the inhibition-associated phospho-Ser9-GSK3B.  
2) Lithium treatment increased the phospho-Ser9-GSK3B both in cells and in mouse brain after chronic administration. |
| Zhang, Phiel, Spece, Gurvich, & Klein, 2003 [28] | Pharmacological study | 293T cells, Neuro2A, and NIH3T3 cells from American Type Culture Collection- treated with lithium or VPA (varying concentration and duration) | Evaluated the effect of lithium or valproate on GSK3B. | 1) Lithium treatment inhibited GSK3B activity which was mediated through increased phospho-Ser9-GSK3Bin cells.  
2) VPA was not reported to inhibit GSK3B & did not induce increase in phosphorylation of GSK3B. |
| Jonathan Ryves, Dalton, Harwood, & Williams, 2005 [29] | Pharmacological study- using rat primary cells | Rat neocortical neurons – treated with 3mM lithium or 1.8mM valproic acid (VPA). | To examine the effect of lithium and VPA on GSK3B protein levels. | 1) No effect of lithium and VPA on GSK3B protein level. |
| Abdul A, De Silva B, & Gary R, 2018 [30] | Pharmacological study | NIH-3T3 mouse embryo fibroblast, A172 human glioblastoma | Evaluated the effect of lithium or beryllium on phosphorylation of GSK3B and its substrate. | Lithium (20mM) and beryllium (30 & 100 uM) decreases phosphorylation of glycogen synthase (GS) and increased phosphorylation of Ser9-GSK3B in NIH3T3. No change in levels of total GS or GSK3B. |
| Authors            | Study Type                        | Participants                                    | Key Findings                                                                                     | Notes                                                                 |
|--------------------|-----------------------------------|-------------------------------------------------|--------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| Xiaohua Li et al., 2007 [31] | Case-control study (protein level) using PBMCs of subjects and in vitro study by lithium treatment of PBMCs | 23 HC, 9 lithium treated BD and 13 lithium free BD subjects of Caucasian population. | Evaluated change in serine phosphorylation of GSK3B in PBMCs of BD and HC subjects at baseline and in vitro treatment with lithium (20mMol/L) for 1 hour | 1) Basal level of phospho-Ser9-GSK3B was reported to be lowest in HC followed by 3-fold increase in lithium free BD and highest in lithium treated BD subjects. 2) In vitro lithium treatment was also associated with elevation of phospho-Ser9-GSK3B level. 3) No change in total GSK3B protein level. |
| Mendes et al., 2009 [32] | Animal model study (gene expression)–lithium treatment using Wistar rat | 1) In vitro: cortical and hippocampal neurons- 5days LiCl treatment (0.02 to 2mM) 2) In vivo: 12 rats- (0.12mmol lithium; 0.24mmol lithium) | To test the role of role of GSK3B in response to lithium treatment. | 1) In vitro: GSK3B mRNA level was reduced in hippocampal neurons on treatment but no changes in cortical neurons. 2) In vivo: GSK3B mRNA reduced in hippocampus but not in cortex or in leukocyte of treated rats. |
| McCarthy et al., 2011 [33] | Pharmacogenetic study- gene expression in LCLs | LCLs from BD lithium responders (N=13) and lithium non-responders (N=18) | To test the effect of lithium 1mM for 72 hours on GSK3B expression in LCLS from BD lithium response patients | No effect on GSK3B gene expression in both the BD lithium response groups |
| Geoffroy et al., 2017 [34] | Pharmacogenetic study- gene expression in LCLs | 38 French Caucasian BD patients which included 16 ER and 20 NR of lithium. | To test the effect of lithium 1mM (2 -8 days) on GSK3B expression in LCLS from BD lithium response patients | Only on day 8 lithium significantly increased GSK3B gene expression in BD lithium non-responders |
| Benedetti et al., 2005 [35] | Pharmacogenetic association study | 88 BD patients- 2 years on lithium. | To test the association of the GSK3B -50T/C SNP with therapeutic response to lithium. | Mutant allele C carriers improved recurrent rate of mood episode after 2 years on lithium treatment. |
| Szczepankiewicz, | Pharmacogenetic association study | 89 polish BD patients-5 years on lithium | To test the association of GSK3B -50T/C SNP with | No significant differences in genotypic and allelic frequencies between the SNP |
| Study Authors          | Study Type                        | Sample Description                  | Objective                                                                 | Findings                                                                 |
|------------------------|-----------------------------------|-------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Rybakowski et al., 2006 [36] | Pharmacogenetic association study | therapeutic response to lithium.    | and the degree of lithium response.                                       |                                                                          |
| Numajiri et al., 2012 [37]  | Case-control and Pharmacogenetic association study | 29 Japanese BD patients            | To test the association of the SNP with therapeutic response to lithium. | T allele significantly associated with lithium responders.               |
| Y. F. Lin, Huang, & Liu, 2013 [38] | Case-control and Pharmacogenetic association study | 138 Taiwanese BD patients and 131 controls. 83 patients out of 138 cases were evaluated for lithium treatment (24 months) efficacy. | To test the association of the SNP with BD risk and therapeutic response to lithium treatment. | 1) No significant association of the GSK3B - 50T/C SNP and BD. 2) TT genotype was associated with poor lithium treatment response. |
| Iwahashi et al., 2014 [39]  | Pharmacogenetic association study | 42 Japanese patients: 27 were lithium responders and 15 were non-responders. | To test the association of SNP with lithium treatment response.           | No significant difference was reported in genotype and allele frequency of the SNP between lithium responders and non-responders. However, haplotype blocks T-A and C-A (with another SNP [-1727A/T]) was reported to be associated with higher lithium response and lower lithium response respectively. |
| Mitjans et al., 2015 [40]  | Pharmacogenetic association study | Total 131 BD patients from Barcelona which included 26 excellent responders (ER); 62 partial responders (PR) and 43 non-responders (NR) based on lithium response. | To test the association of SNP with lithium treatment response.           | No significant difference was reported in genotype and allele distribution between the lithium response groups. However haplotype rs1732170-rs11921360-rs34558 was associated with lithium response. The C-C-A haploblock was significantly less frequent in group of lithium partial and non-responders than excellent responders. |
| Yang, Van Dongen, Wang, 2015 | Case-control association study | 2 set of fibroblast samples from Corriel Cell | To study the expression of core clock genes in BD and | Set-I: No difference in circadian period. Amplitude of rhythmic expression of NR1D1 |
| Authors                          | Study Type                        | Experimental Details                                                                 | Results                                                                                           |
|---------------------------------|-----------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| Berrettini, & Bućan, 2009 [41]  | Repositories. Set-I: 12BD &12 HC; Set-II: 18BD & 35HC. | HC.                                                                                  | Reduced expression of NR1D1 reduced in BD though statistically not significant. Set-II: GSK3B mRNA & protein level no significant difference between BD and HC, whereas serine-9-phospho GSK3B was significantly reduced in BD. |
| Nováková, Praško, Látalová, Sládek, & Sumová, 2015 [42] | Case-control association study | Buccal cells from 19 HC, 22 BD depressive & 19 BD in manic subjects from Czech Republic. | To investigate the NR1D1 expression profiling for 24 hours in buccal cells of BD and HC. NR1D1 expression profiling for 24 hours in buccal cells. NR1D1 expression profiles of BD in mania was advanced compared to depression and trend advanced compared to control. Amplitude NR1D1 expression higher in mania. |
| Warburton et al., 2015 [43]    | Pharmacological study             | Human SH-SY5Y neuroblastoma cells                                                      | Lithium treatment of neuroblastoma cells showed trend change in NR1D1 expression, whereas valproate did not alter the expression. |
| McCarthy et al., 2011 [33]     | Pharmacogenetic study             | Genetic association: 282 BD Caucasian origin (148 lithium responders and 134 non-responders. LCL experiment: 13 responders and 18 non-responders (1mM lithium for 72hours treatment) | To test the role of NR1D1 lithium treatment response. 1) Allele A at rs2071427 SNP associated with lithium good response. 2) Homozygous allele A at the SNP decreased NR1D1 mRNA (full length transcript) after lithium treatment compared to homozygous G allele. 3) AA genotype was associated with trend increase of NR1D1 (both full and truncated transcript) mRNA compared to GG after treatment. |
| Geoffroy et al., 2017 [34]     | Pharmacogenetic study             | LCLs from 36 BD subjects (20 lithium responders and 16 non-responders of Caucasian origin) | To analyse the gene expression of BD LCLs at day2,4,8 on 1mM lithium treatment. NR1D1 gene expression was downregulated at day 2 in responders. NR1D1 was upregulated at day 4 for both responders and non-responders. No significant changes at day 8. |
Campos-de-Sousa et al., 2010 [44]; Kishi et al., 2008 [45]; Kripke, Nievergelt, Joo, Shekhtman, & Kelsoe, 2009 [46]; Severino et al., 2009 [47].

| Case-control candidate gene association studies | BD and HC subjects in various studies | Tested the association of different NR1D1 polymorphisms with risk of BD | The studies have reported positive association of the NR1D1 polymorphisms with risk for BD. |

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**Supplementary table 5: Reagents and antibodies**

1. Anti-Nestin (Life technologies, Cat # A24354)
2. Anti-Pax6 (Sigma-Aldrich, Cat # AB2237)
3. Anti-TOMM22, (Sigma-Aldrich, Cat # HPA003037)
4. B27 supplement without Vitamin A (Thermofisher-Gibco, Cat #12587-010)
5. Beta-mercaptoethanol (Thermofisher-Gibco, Cat # 21985-023)
6. BFGF (Thermofisher-Gibco, Cat # PHG6015)
7. Carbonyl cyanide m-chorophenyl hydrazone (CCCP) (Sigma-Aldrich, Cat # C2759)
8. Click-it Edu Alexa Fluor 488 imaging kit (Thermofisher-Invitrogen, Cat # C10337)
9. DAPI (40, 6-diamidino-2-phenylindole) (Thermofisher-Life Technologies, Cat # R37606).
10. DMEM/F12 (Thermofisher-Gibco, Cat #10565-018)
11. DNA isolation kit (Macherey-Nagel, Cat # 740951.50)
12. Fetal bovine serum (Thermofisher-Gibco, Cat # 10270106)
13. Glutamax (Thermofisher-Gibco, Cat # 35050-061)
14. Heparin (Sigma-Aldrich, Cat #H3149)
15. Knockout DMEM (Thermofisher-Gibco, Cat # 10829-018)
16. KOSR (Thermofisher-Gibco, Cat # 10828-028)
17. Lithium Chloride (Sigma-Aldrich, Cat # L7026)
18. Lonza Mycoplasma detection kit (Lonza, Cat # LT07-318)
19. Matrigel (Corning, Cat. #354277)
20. MitoTracker™ Deep Red FM (Thermofisher-Invitrogen, Cat # M22426)
21. N2 supplement (Thermofisher-Gibco, Cat #17502-048)
22. Non-Essential Amino Acids (Thermofisher-Gibco, Cat # 11140-050)
23. Paraformaldehyde (Sigma-Aldrich, Cat # P6148)
24. Penicillin-Streptomycin (Thermofisher-Invitrogen, Cat # 15140-122)
25. Propidium Iodide dye (Thermofisher-Invitrogen, Cat # P3566)
26. Real Time PCR (q-PCR) system (Thermofisher, Cat # AB7500)
27. RNase A (Thermofisher-Invitrogen, Cat # 12091021)
28. RPMI-1640 (Himedia, Cat # AL060A)
29. Secondary antibody Alexa flour 488 donkey anti-mouse (Life Technologies, Cat # A24350)
30. Secondary antibody Alexa flour 594 donkey anti-rabbit (Life Technologies, Cat # A24343)
31. StemPro Accutase (Thermofisher-Gibco, Cat # A1110501)
32. SuperScript™ VILO™ cDNA Synthesis Kit (Thermofisher-Invitrogen, Cat # 11754050)
33. Sytox Green (Thermofisher-Invitrogen, Cat # S7020)
34. Taqman gene expression -housekeeping gene assays (Thermofisher-Applied Biosystems, Cat # 4448485)
35. Taqman gene expression master mix (Thermofisher-Applied Biosystems, Cat # 4369016)
36. Taqman gene expression -target gene Assays (Thermofisher-Applied Biosystems, Cat # 4331182)
37. Triton X-100 (Invitrogen, Cat # A24352)
38. Trizol (Thermofisher-Ambion, # 15596-026)
39. Valproic acid sodium salt (Sigma-Aldrich, Cat # P4543)
40. Vectashield (Vector labs, Cat # H-1000)
41. Verapamil (Sigma-Aldrich, Cat # V4629)