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Unraveling the biology of bipolar disorder using iPS derived neurons

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Abstract

Objectives—Bipolar disorder has been studied from numerous angles, including pathological studies to large scale genomic studies, overall making moderate gains toward an understanding of the disorder. With the advancement of induced pluripotent stem (iPS) cell technology, *in vitro* models based on patient samples are now available that inherently incorporate the complex genetic variants that largely are the basis for this disorder. A number of groups are starting to apply iPS technology to the study of bipolar disorder.

Methods—We selectively reviewed the literature related to understanding bipolar disorder based on using neurons derived from induced pluripotent stem cells.

Results—So far work has primarily used the prototypical iPS cells. However others have been able to transdifferentiate fibroblasts directly to neurons. Others still have utilized olfactory epithelium tissue as a source of neural-like cells that do not need reprogramming. In general, iPS and related cells can be used for studies of disease pathology, drug discovery, or stem cell therapy.

Conclusions—Published studies have primarily focused on understanding bipolar disorder pathology, but initial work is also being done to use iPS technology for drug discovery. In terms of disease pathology, some evidence is pointing toward a differentiation defect with more ventral cell types being prominent. Additionally, there is evidence for a calcium signaling defect, a finding that builds on the genome-wide association study results. Continued work with iPS cells will certainly help us understand bipolar disorder and provide a way forward for improved treatments.

Keywords

bipolar disorder; induced pluripotent stem cells; iPS cells

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Background on Bipolar Disorder Pathophysiology

Efforts to understand the etiology and pathology of bipolar disorder have ranged from genetic studies to post-mortem work, neuroimaging, animal models, and now induced pluripotent stem (iPS) cells. It has long been known that a family history of bipolar disorder puts one at risk for developing the disorder. Family, twin, and adoption studies have all shown the disorder to be largely genetically heritable, with estimates from twin studies being as high 93% (1). With the latest large-scale genome-wide association studies (GWAS), 25–30% of the heritability has been captured (2). (In these studies, the results are finding particular variations of common SNPs that are present in bipolar disorder individuals at a greater frequency than in controls. While these variations of the SNPs are likely not causative genetic changes, they are likely linked to some nearby unknown genetic change that presumably is causative.) With these studies, common genetic variants with allele frequencies greater than or equal to 5% can be examined. Detecting more rare variants will likely require whole genome sequencing. Other approaches to understanding the illness, such as post-mortem and imaging studies have had varied results of unclear significance making it difficult to draw larger conclusions. Most animal models of either depressive-like or mania-like behavior are based on over or underexpression of specific genes in mice, failing to capture the genetic complexity, the spontaneous cyclical nature of the episodes, or the specific behaviors of individuals with bipolar disorder. One example from the interesting findings so far revolves around voltage gated calcium channels. These channels have been associated with bipolar disorder but also schizophrenia in GWAS experiments. More recent work with iPS cells has also shown that, at least in some experimental paradigms, bipolar iPS-neurons have hyperactive calcium signaling events that even normalize with lithium, but only in cells from patients that also respond to lithium (3). Overall the data support a heritable complex genetic disorder with hundreds of genes contributing and being the predominant cause of bipolar disorder. While it has been challenging making progress toward an understanding of bipolar disorder, experimental tools are becoming more powerful and comprehensive.

While the genetic studies have been incredibly informative and pointed further experiments in the direction of certain signaling pathways and ion channels, it has proven difficult to know how to experimentally model a complex set of genetic risk alleles. Furthermore, post-mortem work, while definitely reflective of disease processes, also represent the effects of drug treatments as well as secondary disease process effects. It is also an intractable model for testing experimental variables such as drug effects or altering genes of interest. Experiments working with clinical samples also have their own challenges, often limited in their ability to examine neural tissue, readily alter treatment with mood stabilizing drugs, or formally test the function and biology of neurons. Ideally bipolar disorder could be modeled in a way that captures its complex genetic background, allows disease pathology to be experimentally dissected, permits novel drugs to be tested, and accurately represents what is occurring *in vivo*.

Background on iPS Cells

The development of human induced pluripotent stem cells opened up vast realms for novel research. In 2007, Takahashi et al. determined four critical factors for reprogramming cells into iPS cells, Oct3/4, Sox2, c-Myc, and Klf4 (4). When these factors, now known as the Yamanaka factors, were expressed in somatic cells, a small fraction of these cells would successfully be reset (a.k.a. induced) to an embryonic state that could then be differentiated into endoderm, mesoderm, or ectoderm cells like an actual embryonic stem cell. While their full potential is still being determined, the key research opportunities with iPS cells fit into three broad categories. The first is the study of disease pathology, an especially critical opportunity for neurological and psychiatric disorders where live, pathological tissue is not readily available. Next is the study of drug response and development with iPS cells providing a tractable and scalable system. Finally, therapeutic applications of iPS cells are in the early stages of investigation.

Cellular Sources of iPS Cells and Related Alternatives

Another important aspect in regard to the potential of iPS cells is the cellular starting material. The original cellular source for iPS cells was fibroblasts. They have also been the mostly widely used cell type; however, they do require a skin biopsy making the source material somewhat more difficult to obtain. Lymphoblastoid cell lines are widely available and numerous repositories exist. Barrett et al. demonstrated that lymphoblast-based iPS cells could be generated from a variety of control and disease sources (5). Lymphocytes are collected from blood samples and converted to a proliferative lymphoblastoid cell line using Epstein Barr virus (EBV). A major advantage of this source is the ease of collection of samples as well as existing large cell banks of lymphoblastoids that have been characterized from the clinical to molecular level. Using lymphoblastoid cells is an exciting new direction because of the available samples. Though more work is necessary, it may allow studies of a much broader range of subjects.

Additionally, there are alternatives to iPS cells, for example induced neurons (iN) (alternatives summarized in Table 1). In 2010, Vierbuchen et al., investigated a group of 19 factors involved in neuronal development and epigenetic remodeling to determine if it was possible to directly convert mouse fibroblasts into functional neurons (6). Through a series of experiments using different combinations of factors, they were able to find that the expression of the single factor *Ascl1* was sufficient to induce immature neuronal features, and that by adding in *Brn2* and *Myt1l*, mature iN could be generated at an efficiency of around 20%. Immunohistochemistry and electrophysiology experiments showed that these mature iN expressed neuronal markers such as *Tuj1*, *NeuN*, and *MAP2*, as well as showing neuronal activity, such as action potentials and synapse formation. More recently, their group has continued to work on the details of the steps involved in direct reprogramming (7) as well as a generation of oligodendrocyte precursors in rats and mice (8).

Another alternative to iPS cells is olfactory neurons, which can provide an accessible source of neural tissue. Olfactory epithelium contains neuronal stem cells and basal precursor cells that continue to produce olfactory neurons. Unlike iPS cells, no exogenous factors are

required nor differentiation factors. Furthermore, this tissue is readily biopsied under local anesthesia. Horiuchi et al. tested an important hypothesis about whether olfactory neurons are a good surrogate for CNS tissue (9). They investigated this by comparing the expression profiles of olfactory neurons with lymphoblasts, iPS cells, mesenchymal stem cells, embryonic stem cells, fetal and adult brain tissue. Principle components analysis (PCA) found that, at an exploratory level of analysis, olfactory neurons appear most closely related to the mesenchymal stem cells versus iPS and embryonic stem cells or neurons produced from these sources, and most different from lymphoblasts. So olfactory neurons may be a reasonable model for CNS neurons, having the advantage of less exogenous factors but, based on expression data, seeming to less closely resemble CNS neurons than neurons from iPS cells.

In summary, there are now multiple cell sources for deriving iPS and other alternative cell types that can be used to study neural cells *in vitro*. They vary in how precisely they represent the cell type of interest. Some retain more markers of the source tissue, and they can also vary in their differentiation potential. Keeping in mind the cellular source and how they were reprogrammed is an important experimental factor to keep in mind when planning and evaluating experiments.

Studies of Bipolar Disorder

Below we will provide a review of the studies published so far using iPS cells to gain further insight into bipolar disorder. The review has been grouped by the themes of the findings so far, discussing the relevant data from each paper behind each theme. When a paper is first introduced, a general overview of that paper is given. Here, it is worth providing a general overview of the differences between the papers (see Table 2). In general, there is a significant amount of variation in the differentiation protocols, with some studies making more differentiated neurons, such as forebrain or dentate gyrus granule cells. Additionally some studies looked at early neurons, just 2 weeks old whereas others allowed them to mature until 12 weeks. Wang et al. were able to directly induce neurons from fibroblasts (10), saving a significant amount of time in the challenging process of reprogramming fibroblasts, but this may not have been as accurate of a model. Additionally, the sample numbers generally tended to be low, at most 12 disease versus 6 controls. Another important difference is sample selection; as bipolar disorder is largely a complex genetic disorder, some efforts were made to focus on a clinical phenotype (such as using lithium-responsive samples) or on a more similar genetic profile (such as studying bipolar disorder within a family or larger pedigree), all strategies worth considering to improve the chance of making meaningful discoveries. Another potentially important difference is the age of the iPS neurons. In many ways they likely appear young like an early embryonic neuron, but they may also reflect the age of the patient, such as having telomere shortening. Some studies look at these neurons months after differentiation to examine more mature synaptic development. In general, many of the BP iPS neurons underwent expression profiling and some morphological characterizations. From there, some studies branched out to further look for differences such as electrophysiological changes or differences in mitochondrial function. Ultimately, it is important to take an open-minded approach to studying bipolar

disorder with iPS neurons. It is not yet known which neuronal subtypes are critical for the disease pathology nor what is the ideal cellular model for studying them.

Using Bipolar iPS Cells for Drug Screening Applications

A cellular model of bipolar disorder would allow for high throughput screening and drug discovery. Cellular models allow for rapid screening of FDA approved drugs that may have new applications, naturally occurring compounds from traditional medicine, as well as novel compounds. Additionally, some type of cellular phenotype thought to be important in the disease process must be monitored as different drugs are tried. Wang et al. have made good progress towards finding a bipolar cellular phenotype that is suitable to a high throughput drug screening assay (10).

Wang. et al generated directly induced (transdifferentiated) neuronal-like cells (iNLC) from the fibroblasts of patients that were lithium responsive or non-responsive (10). While distinct from iPS cells, the iNLC allowed more rapid generation, just 14 days, of neuronal like cells, morphologically suggesting they were forming axons and dendrites and histologically showing markers of neural stem cells and neurons, but also still expressing fibroblast markers. Wang et al. then used these cells with a novel optical imaging platform known as the BIND Scanner. Cells are plated on a BIND biosensor that has a grating structure that allows light to resonate and reflect in a precise manner. Changes in cellular mass close to the sensor create a shift in the resonated, reflected light giving an optical readout, the peak wavelength value (PWV), for cell attachment. Changes in PWV are thought to be a measure of changes in adhesion, and they've shown that changes in integrin-extracellular matrix interactions, for example, can be measured by monitoring PWV. Additionally, cell morphology could be imaged including parameters like cell count, size, and fraction of the sensor covered. Ultimately differences were not seen regarding cell count, size, or fraction. However, an interesting difference appeared between lithium responsive and non-responsive iNLCs, the responsive iNLCs showed a significantly greater increase in PWV, in other words adhering more strongly to the biosensor. Control iNLCs were intermediate. This change was not explained by other co-variables such as sex, lithium treatment status of the patient, or even cell count. It did not respond to acute treatment with lithium in the experimental model over the 5 hours they were imaged. In summary a clinically associated phenotype, change in cellular adhesion, was identified in bipolar patients that had a history of responding to lithium. The BIND platform could allow characterization of patient samples for clinical diagnostics or the rapid screening of new therapeutics. Further validation of the iNLC model is important to gain confidence that they reflect pathologic neurons from bipolar patients.

Developmental Defects

A major question that can hopefully be addressed with iPS neurons is whether there is a developmental problem in the bipolar brain leading to the disorder. This has been suggested by other approaches, such as imaging or postmortem work. Imaging studies of adolescent cases of bipolar disorder have revealed changes in the amygdala and prefrontal cortex, sometimes differing from what is seen in adults with bipolar disorder, reviewed in (11). Furthermore, a recent longitudinal study also showed gray matter loss and diminished white

matter growth in the insula and prefrontal cortex (12). Postmortem studies have shown changes with neuronal and glial number and size in areas such as the dorsolateral prefrontal cortex and the hippocampus, postmortem studies reviewed in (13). Overall these imaging studies point to a developmental etiology that is ongoing when symptoms commonly first occur. O'shea and McInnis clearly posited that there is a developmental origin underlying bipolar disorder, noting problems with “organizational and neuronal migration alterations” that require a “developmental model” such as iPS cells (14).

Chen et al. published the first work studying bipolar disorder with iPS cells and shed some light on these questions (15). They generated iPS cells from 3 individuals with bipolar disorder and 3 controls. Next these were differentiated into neurons that largely resembled forebrain neurons. These were characterized chiefly through microarray expression studies and careful pathway analysis including KEGG pathways and Gene Ontology terms. Comparing the neurons to the iPS cells, bipolar neurons had increased expression of genes important for ventral neurons, such as NKX2-1, whereas control neurons had increased expression of genes important for dorsal neurons, such as EMX2. Numerous other genes important for early neuronal differentiation and patterning fit with this general trend. Possibly pathologic changes began at an early stage, hinted at by differences between the BP and control iPS cells, including changes to genes involved with proliferation, regulation, and differentiation, e.g. transforming growth factor- β -related transcripts being expressed less in BP iPS cells. They hypothesize that lithium therapy may help remedy these defects by activating WNT pathways that can help dorsalize neural stem cells.

Work from Madison et al. also suggests that cellular proliferation may be altered in bipolar disorder (16). They explored BP iPS cells using a novel experimental paradigm. As bipolar disorder is a complex genetic disorder with quite possibly different complex genetic forms of the disorder, they attempted to eliminate this variable by studying bipolar disorder within a family, choosing 2 affected siblings and their unaffected parents as the subjects. However, as bipolar disorder is highly heritable, at least 80% and individuals usually have a family history of mood disorder, this raises the difficult question of whether the unaffected parents did not have the critical genetic insults, thus being able to serve as appropriate controls, or had they somehow only avoided the environmental insult, thus having quite a similar genetic risk for bipolar disorder and making poor genetic controls. Likely to shed some light on this, whole-genome SNP profiling was performed. In terms of risk alleles for bipolar disorder, one interesting association was reported. The mother was heterozygous for a CACNA1C risk allele. CNVs were also analyzed and no large (<10 kb) de novo CNVs were uniquely found in both affected siblings. In terms of understanding differentiation potential, the iPS cells were exposed to factors leading to a general neural differentiation producing both central and peripheral neurons. Immunostaining for CXC chemokine receptor 4, a marker of central neural progenitors, as well as a peripheral marker, showed that bipolar iPS cells had a lower propensity for generating central neural progenitors. Additionally the bipolar central neural progenitors had lower rates of proliferation as measured by BrdU incorporation. Overall, it seems that iPS bipolar neurons have some decreased ability to generate central neural progenitors, and according to Chet et al. possibly a specific problem with more dorsal neuron types (15).

Changes in Electrical Activity

More and more data is pointing toward aberrant calcium signaling in bipolar neurons. Calcium abnormalities were first reported in platelets by Dubovsky et. al (17). GWAS results have also highlighted numerous L-type calcium channel subunits strongly suggesting that calcium is dysregulated in some manner in bipolar disorder. iPS neurons provide an excellent way to study calcium signaling and carefully investigate the electrical properties of specific neuron types in bipolar disorder. In the study by Chen et al. where they generated forebrain neurons, they allowed the neurons to mature in culture for 12 weeks (15). At this point, they measured calcium transients and wave amplitude, noting that they were decreased in BP neurons pre-treated with lithium. In Madison et al's work, they also characterized RNA expression in the 6 week old neurons (central neural progenitor cells, TuJ1 and MAP2 positive) (16). Interestingly, 5 of the differentially expressed genes were related to calcium channels, some with increased expression, some with decreased.

Recently, Mertens et al. studied 3 week old dentate granule-like neurons (produced from iPS cells), from 3 lithium-responsive bipolar individuals, 3 non-responsive bipolar individuals, and 4 controls (3). After transcriptome studies suggested aberrant electrical properties in the cases, they performed patch clamp experiments noticing a general theme of hyperexcitability. Specifically there was increased sodium channel activation in neurons from bipolar individuals. Additionally action potentials were altered with lower thresholds, increased numbers of evoked potentials, increased maximal amplitudes, and higher spontaneous frequencies. When they looked at network activity through monitoring a fluorescent calcium indicator, they similarly saw increased calcium events. Most interestingly, they saw these in both the lithium responsive and non-responsive neurons. However, when treated with lithium for 1 week, some aspects of the hyperexcitability as well as the calcium events were reduced only in the neurons from lithium-responsive patients. Also, when these neurons were allowed to mature more, the hyperexcitability reversed.

Mitochondrial Defects

Mitochondrial dysfunction also may play a role in bipolar etiology. Quiroz et al. discussed the range of evidence suggesting how mitochondrial problems may interface with calcium signaling, handling oxidative stress, and even synaptic plasticity (18). In this context, Mertens et al. investigated mitochondrial function, studying mitochondrial membrane potential (3). They noticed “enhanced mitochondrial function.” Additionally, the size of the mitochondria was reduced. This reduced size has been associated with increased mitochondrial transport possibly helping induce the increased neuronal activity discussed above. Supporting this hypothesis, lithium treatment, rescued the mitochondrial size phenotype only in the lithium-responsive samples.

Gene Expression

Many of the publications reviewed above have also thoroughly examined RNA expression through microarrays or RNA-seq studies. Each of the studies used different cell types, with a

different experimental paradigm, and unique differentiation protocols. Nevertheless, it's useful to consider the general findings from RNA studies. Madison et al. used both nanostring sequencing and RNA-seq, finding some genes differentially expressed, such as PAX6 – playing a role in neurogenesis, and CACNA1C and FGF14 – with roles in regulating sodium and calcium channel function (16). Mertens et al. also used RNA-seq to characterize the dentate granule-like neurons, finding genes related to increased mitochondrial function, calcium signaling, and ligand-receptor function, protein kinase A and C function, and genes important for action potentials (3). They posited that increased mitochondrial function as well as increased PKA/PKC activity may be related to the increased action potentials seen.

Bavamian et al. specifically investigated expression levels of miR-34a, a microRNA that is thought to play a role in neural development and whose levels are affected by the mood stabilizers lithium and valproic acid (19). They first investigated this in post-mortem cerebellum samples, and noticed increased levels of miR-34a in bipolar samples. They wanted to eliminate potential biases from working with post-mortem tissues, so they also investigated this in both iPS-neural progenitors and differentiated neurons. They worked with just 1 bipolar sample and 1 control sample, but did see a consistent trend of higher miR-34a levels in the bipolar individual. To expand their sample numbers, they also examined this in induced neurons, made from fibroblasts, and found the same effect, supporting both their finding and the use of induced neurons. They then identified putative miR-34a targets that overlap with genes from the bipolar GWAS studies. Through a variety of expression correlation studies, miR-34a overexpression, anti-miR-34a expression, and studies of neuron morphology, they showed data supporting miR-34a differential upregulation in bipolar disorder, decreasing amounts of ANK3 and CACNB3, also associated with limited neuronal differentiation.

Another recent study focuses on microarray expression data from iPS neurons from an Old Order Amish pedigree (20). They compared 4 affected sibs and cousins with 4 controls, at the neural progenitor stage, 2-week-old “early” neurons, and 4-week-old “late” neurons. The expression data when examined through principle components analysis for similarity found that the bipolar and control samples were not different on a global scale, but that the samples still separated based on the stages above. They did find differentially expressed genes between bipolar and controls at the late stage. When compared based on gene networks, such as gene ontology analysis, they found differences in metabolic processes, protein transport, WNT pathway, and GSK3beta.

Conclusion

The early work using iPS cells to study bipolar disorder has shown some promising findings. It seems likely that there are some differentiation deficits in bipolar disorder with Chen et al. seeing a more ventral phenotype in their “forebrain” neurons (15) and Madison et al. also noticing a proliferation deficit (16). Additionally, abnormal calcium signaling suggested by genome wide association studies, can now be demonstrated at the cellular level, and may be corrected in some situations by the drugs used to treat bipolar disorder (15,16). It is unclear at this point how the altered differentiation potential and calcium signaling relate. While

these could be separate consequences of some causative pathology, it's also possible that altered neuronal differentiation could be affecting the way these neurons carry out neuronal "functions" such as calcium currents. Alternatively, abnormal calcium currents may be limiting the differentiation potential of these cells from an early stage.

A number of limitations of iPS cells need to be addressed in making conclusions from any experiment. Many of the challenges revolve around the iPS technology itself. iPS clones made from the same individual donor can demonstrate genetic differences as well as varied differentiation potential. Genetic differences can be induced by viral expression of the Yamanaka factors, so non-genome integrating expression methods have been developed (see reference (21) for a comparison). Additionally, as is apparent in the above studies, sample numbers are often small limiting the power of these studies. Thus, efforts to reduce statistical noise can enhance the ability of iPS studies to find meaningful results. Examples include using family members as controls as done by Madison et al. (16) or by examining likely subtypes of disease, such as lithium responders (3). Finally, the differences detected in iPS-derived cells from patients with bipolar disorder, or another disorder for that matter, still do not equate to *in vivo* definitive pathology. The neurons derived from iPS cells seem to best reflect fetal to early developmental stages of neurons, but not mature neurons that operate within a neural network nor that have undergone the range of environmental stressors. Still, these iPS-derived neurons offer an incredible window into the human brain. With careful correlation of cellular phenotypes to clinical phenotypes, and correction with drug rescue, we can have a very versatile model capturing disease pathology.

However, iPS cells will allow us to make progress in understanding disease pathology and advancing clinical treatment of patients with bipolar disorder (see Figure 1). Work can now proceed to understand the result of GWAS studies. (For clarity, GWAS results show common SNPs that are more commonly found in cases versus controls. These common SNP "hits" are not thought to generally be causative, but instead some unknown nearby genetic change. Next-generation sequencing techniques are building on this and have started to identify individual novel variants that very well may be causative (22).) Whereas previously long lists of results were analyzed solely through gene networks attempting to understand what cellular systems were affected, the roles of individual genes can be more readily understood. Because bipolar disorder is the result of many genes being affected, likely either mild mutations or changes in gene regulation, it has been inadequate to study a "pathologic" copy in an otherwise healthy cell or animal model. Now we can do experiments in samples from patients that have the complex genetic background that put them at high risk for bipolar disorder. By using gene editing techniques to correct a handful of the most likely critical genes we hope to rescue the putative pathologic cellular phenotypes and show that both the genes and phenotypes play a role in disease pathology. Similarly, we could do experiments on the cells of unaffected family members, that have a high genetic risk for bipolar disorder but in fact have not developed it. By editing in mutations, we hope to cause the bipolar phenotype in iPS-derived neurons. Overall there is great hope that this approach will provide a more complete picture of what is occurring at the cellular level and gain a functional understanding of the pathologic genes implicated in the GWAS results.

Additionally, iPS neurons are an important part of changing how we care for patients with bipolar disorder. One day could we, similar to our colleagues in oncology, run a series of lab tests that show us the particular genetic form of bipolar disorder we are treating? We also look forward to the day when we can similarly provide a prognosis, giving the patient a sense of their risk for manic episodes, and being able to recommend optimal treatments for that individual patient based on pharmacogenetics. We already believe that bipolar disorder is really a collection of different disorder subtypes driven by different genetic pathologies affecting specific cellular systems and ultimately the brain in specific ways. For example, there is a subset of patients, approximately 25% that respond well to lithium. Being able to share with the patient a better understanding of their subtype of bipolar disorder and what specific treatments will work for them is a dream, but it is now a realistic dream. Ultimately, we believe that studying bipolar disorder with iPS cells will be one of the key tools that will help us understand the relation between the complex genetics of bipolar disorder, the cellular pathology, change at the level of brain circuitry, and ultimately provide more effective treatment for individuals with bipolar disorder.

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References

1. Kieseppä T, Partonen T, Haukka J, Kaprio J, Lönngqvist J. High concordance of bipolar I disorder in a nationwide sample of twins. *Am J Psychiatry*. 2004 Oct; 161(10):1814–21. [PubMed: 15465978]
2. Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, Perlis RH, et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat Genet. Nature Research*. 2013 Aug 11; 45(9):984–94.
3. Mertens, J, Wang, Q-W, Kim, Y, Yu, DX, Pham, S, Yang, B. , et al. *Nature*. Vol. 527. Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved; 2015 Oct 28. Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder; 95–9.
4. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007 Nov 30; 131(5):861–72. [PubMed: 18035408]
5. Barrett, R, Ornelas, L, Yeager, N, Mandefro, B, Sahabian, A, Lenaues, L. , et al. *Stem Cells Transl Med*. Vol. 3. AlphaMed Press; 2014 Dec 1. Reliable generation of induced pluripotent stem cells from human lymphoblastoid cell lines; 1429–34.
6. Vierbuchen, T, Ostermeier, A, Pang, ZP, Kokubu, Y, Südhof, TC, Wernig, M. *Nature*. Vol. 463. Macmillan Publishers Limited. All rights reserved; 2010 Feb 25. Direct conversion of fibroblasts to functional neurons by defined factors; 1035–41.
7. Lujan E, Zunder ER, Ng YH, Goronzy IN, Nolan GP, Wernig M. Early reprogramming regulators identified by prospective isolation and mass cytometry. *Nature*. 2015; 521(7552):352–6. [PubMed: 25830878]
8. Yang N, Zuchero JB, Ahlenius H, Marro S, Ng YH, Vierbuchen T, et al. Generation of oligodendroglial cells by direct lineage conversion. *Nat Biotechnol*. 2013; 31(5):434–9. [PubMed: 23584610]
9. Horiuchi Y, Kano S-I, Ishizuka K, Cascella NG, Ishii S, Talbot CC, et al. Olfactory cells via nasal biopsy reflect the developing brain in gene expression profiles: utility and limitation of the surrogate tissues in research for brain disorders. *Neurosci Res*. 2013 Dec; 77(4):247–50. [PubMed: 24120685]

10. Wang, JL, Shamah, SM, Sun, AX, Waldman, ID, Haggarty, SJ, Perlis, RH. Transl Psychiatry. Vol. 4. Macmillan Publishers Limited; 2014 Jan 26. Label-free, live optical imaging of reprogrammed bipolar disorder patient-derived cells reveals a functional correlate of lithium responsiveness; e428
11. Keener MT, Phillips ML. Neuroimaging in bipolar disorder: A critical review of current findings. Curr Psychiatry Rep. 2007; 9(6):512–20. [PubMed: 18221633]
12. Najt, P, Wang, F, Spencer, L, Johnston, JAY, Cox Lippard, ET, Pittman, BP. , et al. Biol Psychiatry. Vol. 79. Elsevier; 2016. Anterior cortical development during adolescence in bipolar disorder; 303–10.
13. Martinowich K, Schloesser RJ, Manji HK. Bipolar disorder: From genes to behavior pathways. J Clin Invest. 2009; 119(4):726–36. [PubMed: 19339764]
14. O'Shea, KS, McInnis, MG. Neuropsychopharmacology. Vol. 40. American College of Neuropsychopharmacology; 2015 Jan. Induced pluripotent stem cell (iPSC) models of bipolar disorder; 248–9.
15. Chen, HM, DeLong, CJ, Bame, M, Rajapakse, I, Herron, TJ, McInnis, MG. , et al. Transl Psychiatry. Vol. 4. Macmillan Publishers Limited; 2014 Jan 25. Transcripts involved in calcium signaling and telencephalic neuronal fate are altered in induced pluripotent stem cells from bipolar disorder patients; e375
16. Madison, JM, Zhou, F, Nigam, a; Hussain, a; Barker, DD, Nehme, R. , et al. Mol Psychiatry. Vol. 20. Macmillan Publishers Limited; 2015 Jun. Characterization of bipolar disorder patient-specific induced pluripotent stem cells from a family reveals neurodevelopmental and mRNA expression abnormalities; 703–17.
17. Dubovsky SL, Lee C, Christiano J, Murphy J. Elevated platelet intracellular calcium concentration in bipolar depression. Biol Psychiatry. 1991; 29(5):441–50. [PubMed: 2018817]
18. Quiroz JA, Gray NA, Kato T, Manji HK. Mitochondrially mediated plasticity in the pathophysiology and treatment of bipolar disorder. Neuropsychopharmacology. 2008; 33(11): 2551–65. [PubMed: 18235426]
19. Bavamian, S, Mellios, N, Lalonde, J, Fass, DM, Wang, J, Sheridan, SD. , et al. Mol Psychiatry. Vol. 20. Macmillan Publishers Limited; 2015 May. Dysregulation of miR-34a links neuronal development to genetic risk factors for bipolar disorder; 573–84.
20. Kim, KH, Liu, J, Sells Galvin, RJ, Dage, JL, Egeland, JA, Smith, RC. , et al. PLoS One. Vol. 10. Public Library of Science; 2015 Jan 10. Transcriptomic Analysis of Induced Pluripotent Stem Cells Derived from Patients with Bipolar Disorder from an Old Order Amish Pedigree; e0142693
21. Schlaeger TM, Daheron L, Brickler TR, Entwisle S, Chan K, Cianci A, et al. A comparison of non-integrating reprogramming methods. Nat Biotechnol. 2015; 33(1):58–63. [PubMed: 25437882]
22. Ament SA, Szelinger S, Glusman G, Ashworth J, Hou L, Akula N, et al. Rare variants in neuronal excitability genes influence risk for bipolar disorder. Proc Natl Acad Sci U S A. 2015; 112(11): 3576–81. [PubMed: 25730879]

Figure 1. Overview of applications of stem cell technology to bipolar disorder

Disease Mechanisms: while iPS cells are still a cellular model and certainly do not fully recapitulate pathology, they do offer a window. For example, Chen et al. (15) utilized gene expression patterns to hypothesize an abundance of ventral cells in BD. **Determining Effects of Variants:** as suggested in the discussion, iPS cells also offer an opportunity to understand the genetic basis of bipolar disorder. Using gene editing techniques, functional studies of genetic variants can be performed. **Diagnostics:** As possible deficits are being found in iPS-neurons, there is the potential to test these phenotypes for their utility as a diagnostic screen. **Drug Discovery:** as in Wang et al. (10) iPS cells can readily serve as the basis for high

throughput drug screening once phenotypes of interest are defined, ideally some aspect of the disease thought to be important for treatment.

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Table 1

Summary of Cell Types

Type	Source	Exogenous Factors	Neuronal similarity	Time to produce
iPS Cells	fibroblasts or lymphoblasts	yes	more similar	slower
iNeurons	fibroblasts	yes	more similar	quicker
Olfactory Neurons	olfactory epithelium	no	less similar	quicker

Table 2

Summary of Studies

Authors	Source	Cell type	#BP v #Ctrl	General design
Chen et al. 2014	iPS	forebrain neurons	3 v 3	disease v control
Wang et al. 2014	iN	neuronal like	12 v 6	disease v control
Madison et al. 2015	iPS	neural progenitor	2 v 2	affected sibs v control parents
Mertens et al. 2015	iPS	DG granule neurons	6 v 4	disease v control
Kim et al. 2015	iPS	neurons	4 v 4	Old order Amish pedigree
Bavamian et al. 2015	iPS and iN	neural progenitor and neurons	1 v 1	disease v control

Authors	Methods	Findings in BP
Chen et al. 2014	RNA microarray, Ca transients	ventralization, abnormal Ca transients
Wang et al. 2014	High-throughput morphology imaging (BIND)	lithium related differences in cellular adhesion
Madison et al. 2015	Expression profiling	proliferation defect, rescued by GSK3 inhibitor
Mertens et al. 2015	RNA sequencing, electrophysiology, mitochondria	increased Ca currents, mitochondrial defects
Kim et al. 2015	Expression profiling	WNT pathway, GSK3 beta changes
Bavamian et al. 2015	QRT-PCR and NanoString	upregulated miR-34a