Title: Generation of Human-induced Pluripotent Stem Cells (hiPSCs)-derived from Dermal Fibroblast of Schizophrenic Patients

Authors: Asghar Parvishan[1,6], Mohammad Taghi Joghataei[2,*], Jafar Kiani[3,4], Ali Shahbazi[1], Faezeh Faghihi[2] ,Mohammad Ghadiri[5]

1. *Department of Neuroscience, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sci-ences, Tehran, Iran.*
2. *Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran.*
3. *Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medi-cal Sciences, Tehran, Iran.*
4. *Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran.*
5. *Psychiatry Center of Iran, Iran University of Medical Sciences, Tehran, Iran.*
6. *Student Research Committee, Iran University of Medical Sciences, Tehran, Iran.*

***Corresponding Author**: Mohammad Taghi Joghataei, Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran. Email: joghataei@iums.ac.ir

To appear in: **Basic and Clinical Neuroscience**

Received date: 2021/09/06

Revised date: 2022/06/21

Accepted date: 2022/07/03

Please cite this article as:

Parvishan, A., Joghataei, M. T., Kiani, J., Shahbazi, A., & Faghihi, F ,Mohammad Ghadiri[5]. (In Press). Generation of Human-induced Pluripotent Stem Cells (hiPSCs)-derived from Dermal Fibroblast of Schizophrenic Patients. *Basic and Clinical Neuroscience*. Just Accepted publication Aug. 15, 2022. Doi: http://dx.doi.org/10.32598/bcn.2022.3697.1

DOI: http://dx.doi.org/10.32598/bcn.2022.3697.1

Abstract

Introduction: Schizophrenia (SCZ) is a psychiatric disorder caused by environmental, social, and genetic factors. This phenomenon is a severe neuropsychiatric disorder with a 1% worldwide prevalence. As SCZ is an exclusively human disorder, animal models cannot mimic all SCZD pathophysiology. Thus, it is crucial to develop a novel human-based specific model of SCZD to elucidate mechanisms of the occurrence of the disease. In this regard, reprogramming somatic cells to human- induced pluripotent stem cells (hiPSCs) serve an expense instrument for modeling SCZ.

Methods: In the present study, we directly reprogrammed the isolated human ear dermal fibroblasts (HDFs) from schizophrenic patients into hiPSCs using some episomal agents in matrigel coated plates. The existence of pluripotency markers was confirmed by the Immunocytochemistry (ICC) test and alkaline phosphatase protocol. We performed karyotype analysis to ensure the maintenance of the normal chromosomes.

Results and Conclusion: Analysis of colonies exhibited intense alkaline phosphatase engagement and represent Oct4, SSEA4, Nanog, and Tra-1-60. HiPSCs show normal karyotype, and they were potent to differentiate to ectoderm, endoderm, and mesoderm. Application of hiPSCs derived from schizophrenic patients would be a promising approach to treating the disease. For checking the behavior of the cells during neurogenesis, we suggest further studies be applied.

Keywords: Schizophrenia, hiPSCs, Fibroblast, Episomal vectors.

Introduction

Schizophrenia disease is an exacting neuropsychiatric disturbance by one percent globally outbreak and 80 – 85% heritability (Sullivan et al., 2003). Multiple reasons, including genetic and environmental factors, have roles in the pathogenesis of schizophrenia (Tsuang et al., 2001), (Palha & Goodman, 2006), and perturbation of these agents could cause disturbance of information of neural network and brain function(Christian et al., 2010). Schizophrenia onset often happens in early adulthood (Sawa & Snyder 2002), but in some patients, the abnormal neurodevelopmental process may start in childhood (Weinberger, 1987), (Marin, 2012). Furthermore, most of the patients show different responses to treatment (Schennach et al., 2012), and only a few percent effectively respond to the treatment (Emsley et al., 2011). Studies have shown that schizophrenia accompanies by abnormal neuronal communication with a significant role for oligodendrocyte and progenitor cells in the pathogenesis (Rubinov, 2013) (Federspiel et al., 2006), (Begre & Konig, 2008). As schizophrenia is an exclusively human disorder, animal models cannot mimic all schizophrenia pathophysiology (Stachowiak et al., 2013), (Jones et al., 2011). Some curative animal model studies have failed in human clinical trials (Thomsen et al., 2010), (Franco & Cedazo-Minguez, 2014). Thus, it is crucial to develop a novel human-

based specific model of schizophrenia to elucidate mechanisms of the occurrence of the disease to find a neoteric therapy. First time in 2007, Yamanaka et al. reprogrammed human fibroblasts to induced pluripotent stem cells(ips) (Takahashi et al., 2007). Some reports shown ips cells could be cultured on mouse embryonic fibroblast as feeders (Ellerstrom et al., 2006; Takahashi et al., 2007), (Crook et al., 2007). Sun and colleagues used matrigel as a feeder-free culture condition to generate individual-specific hiPSCs from the autologous source of cells (Sun et al., 2009). Both human and mouse ips cells could transform to each three germ layers (Maherali et al., 2007), (Meissner et al., 2007), (Takahashi et al., 2007), (Wernig et al., 2007). HiPSCs develop a source of cells genetically similar to the beginning and useful for tissue regeneration and drugs screening. Although, applying viruses to produce hiPSCs has shown a high rate of conveyance utility (Takahashi et al., 2007), (Yu et al., 2007). It can also induce viral pool into the hostess chromosome. Scientists use non-integrating episomal vectors to produce hiPSCs to keep away from accidental genomic pool (Yu et al., 2009).

Considering the importance of developing new approaches to treat SCZD, in our ongoing study, we aimed to produce hiPSCs in schizophrenia patients using episomal vectors to evaluate the treatment efficacy of this method in our future studies.

Materials and Methods

Preparation of human dermal fibroblasts (HDFs)

Human skin biopsies were obtained from dermal punch biopsies of schizophrenia patients following the approval of the ethical committee (Iran University of Medical Sciences, Tehran, Iran; Ethics code: 96-02-87-31209). The diagnosis of schizophrenia was confirmed by psychiatrists' assessment according to SCID-I. All schizophrenia clients were hospitalized in Psychiatry Center of Iran. The earlobe tissue (4 mm) was provided and kept in phosphate-buffered saline (PBS; Medicago, Canada) added by 2% Pen/Strep (Gibco, USA) and then transferred to a cell culture laboratory at Iran University of Medical Sciences. Tissues were minced and then five minutes centrifuged (1200 rpm). Supernatant was give up and tissues were digested by Dispase II (sigma; USA) overnight. After that, the enzyme activity was neutralized using DMEM (Gibco, USA) containing ten percent of FBS (Gibco, USA) then five minutes centrifuged (1200 rpm). After removing supernatant, the tissues were incubated 30 minutes with Collagenase I (Sigma, USA), and Collagenase action was counteracted, as mentioned above. The suspension filtered within a 70μm filter (SPL, China) then five minutes centrifuged (1200 rpm). After removing supernatant, the pellets were cultured in 6- well plates consisting of DMEM added with 10% FBS and 1% pen/strep antibiotics (PAN

Biotech, Germany) and incubated at 37 °C with five percent of CO_2. Three dermal biopsies were used in this study.

Generation of Dermal fibroblast-derived hiPSCs from SCZD Patients

To generate hiPSCs, HDFs were transducted using episomal vectors (pEP4 E02S CK2M EN2L, Addgene) consisting of six markers (OCT-4, SOX-2, KLF-4, c-MYC, Nanog, LIN-28). We produced HiPSCs lines by reprogramming adult skin fibroblasts. Numbers of 5000 HDFs per cm^2 were seeded in each well of a 12-well plate and were transfected one time by using lipofectamine (Invitrogen, L3000-001).

On day 0 or 1, fibroblasts with 70-90 % confluency were infected according to the kit instructions. First, we mixed 2.5 µgr/µl of episomal vectors (Plasmid) and 5µl lipofectamine for 30 minutes in a microtube. Then the solution was added to the cell culture. After 12h, media was replaced.

Six days later, colonies were transferred into matrigel coated plates (1:30; Sigma, USA) containing DMEM/F12, 20% Knockout Serum Replacement, 100 µM non-essential amino acids, 1% pen/ strep, 2 mM L-glutamine (all from Gibco, USA), 100µM ß-mercaptoethanol (Sigma, USA), and 10 ng/ml human basic fibroblast growth factor (b-FGF; PeproTech, USA). Plates were kept at 5% CO_2 with 95% of humidity. For monitor the efficiency effect of transfection protocol, we were used

just 6 columns for hiPSCs reprogramming, while the others were considered as control. After 4 to 5 weeks, colonies were detected. To passage, colonies were rinsed with PBS then keep with DMEM/F12 consist of collagenase I (Sigma, USA) at 37°C for 30 min. Then enzyme was dispelling, and plates were rinsed with PBS. The colonies were moderately removed, pipette out mechanically, then plated in new 24-well plates coated with matrigel. Samples were then tested for immunocytochemical analysis of Tra1–60, Nanog, oct3/4, SSEA4 expression, and alkaline phosphatase staining. At least 3 clones were tested per patient.

The Schematic diagram of research has been shown in figure1.

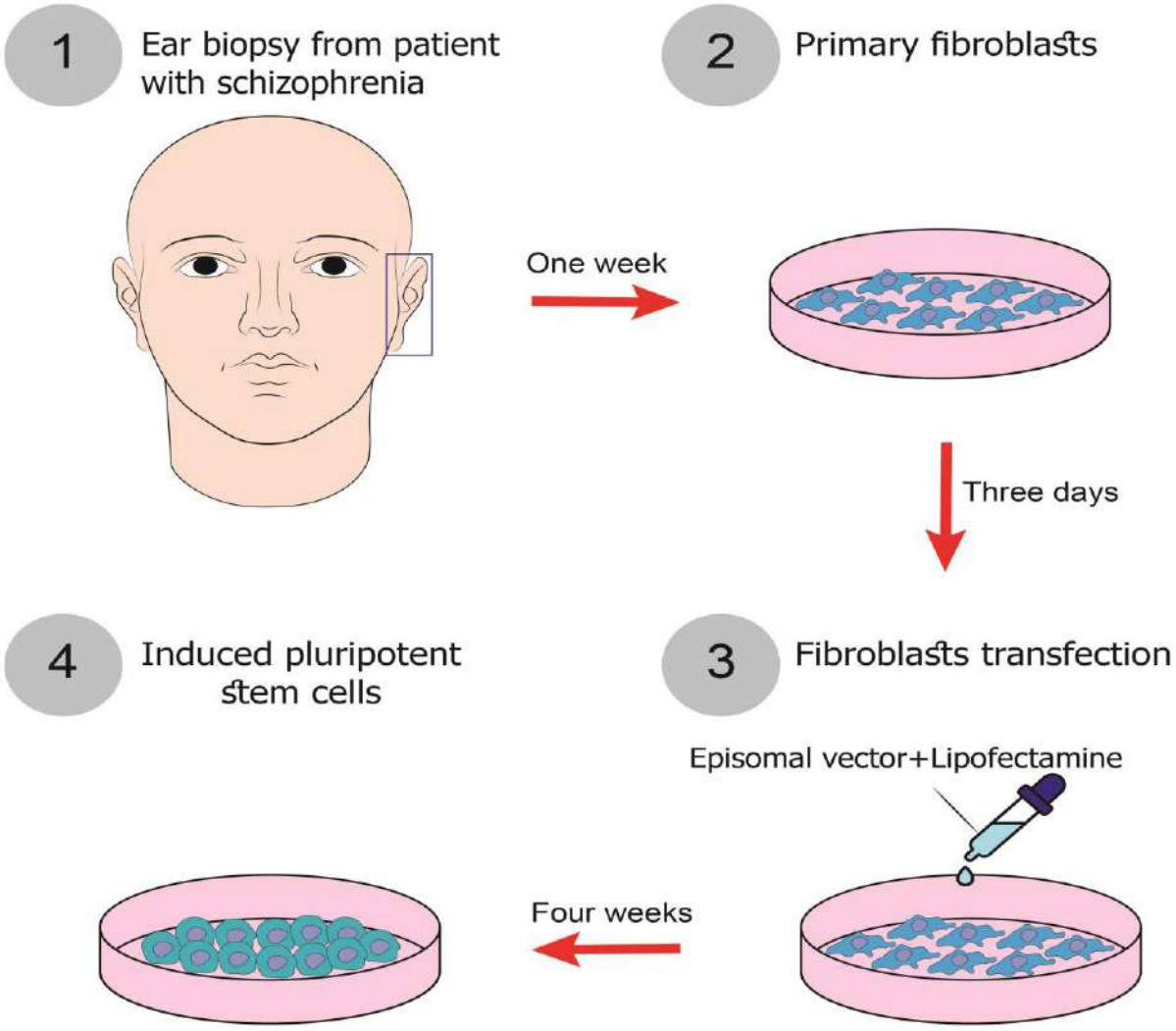

Figure1: the Schematic diagram of research that show all steps.

Characterization of the established hiPSCs

Immunocytochemistry (ICC)

For checking the expression of pluripotency markers, the colonies first twenty minutes were stable by using 4% paraformaldehyde, then penetrated with 0.1–

0.2% of Triton X – 100 for 30 minute. For blocking, 10% mouse serum in PBS was used for 1 hour in 37°C. For analyzing the expression of SSEA-4 and TTRA-1-60, the cells were incubated with Anti-SSEA-4 PE (1:250; Cat. No.CS204438) and anti-TRA-1-60 FITC (1:200; Cat. No. CS204460) antibodies for 30 minutes in a 37ºC, and 5% CO_2 chamber.

To explore the expression of Oct-3/4 and Nanog, Human Pluripotent Stem Cell 3-Color Immunocytochemistry Kit (Cat no. SC021; bio-techne, USA) was used. Briefly, 10% normal donkey serum in 0.3% Triton® X-100 was used as the blocking buffer. After removing the blocking buffer, the cells were stained with NL637-conjugated Goat Anti-Human Oct-3/4 and NL493-conjugated Goat Anti-Human Nanog antibodies.

Derived HiPSCs were differentiated to three germ layers. According to manufacturer's instructions, germ layer differentiation was subsequently verified using Human Pluripotent Stem Cell Functional Identification Kit (Cat. No. SC027B). Goat anti-human Otx2, Goat anti-human Brachyury, and Goat anti-human Sox17 were used as antibodies to verify ectoderm, mesoderm, and endoderm differentiation. Also, we used NL557-conjugated donkey anti-Goat (R&D systems, Cat.No.NL001) as the secondary antibody (red). All nuclei were counterstained with DAPI (blue).

Alkaline phosphatase Test

Alkaline phosphatase test was implementing by applying alkaline phosphatase staining kit II (Reprocell, USA) to evaluate AP-expressing hiPSCs colonies. Concisely, cells were washed with PBST (PBS+ triton, final concentration of 0.05%). Then, they were fixed using the fix solution medium for 2-5 minutes. After that, cells were incubated in fresh prepared AP solution 15 minutes in room temperature. When the color turned bright, reaction was stopped.

Karyotype Analysis

For karyotype analysis, the cells were treated 16 hours with thymidine (Sigma, USA) at 37°C in 5% CO_2. Three hours after washing, the cells were treated with colcemid (Gibco, 0.15 µg/ml, 30 min). separated colonies 16 minutes were exposed to 0.075 M KCl at 37°C then were fixed in ice-cold 3:1 methanol: glacial acetic acid three times and dropped onto pre-cleaned chilled slides. At least 20 metaphase spreads were screened, and ten were evaluated for chromosomal rearrangements.

Differentiation of hiPSCs into Three Germ Layers

To confirm the pluripotency of hiPSC clones, three lineage differentiation assays were performed using Human Pluripotent Stem Cell Functional Identification Kit (Cat. No. SC027B). According to the manufacturer´s instruction, Cells were harvested and stable for analysis of lineage-specific markers on day 5 (for

mesoderm and endoderm lineages) and day 7 (for the ectoderm lineage). Immunofluorescence assay was carried out with antibodies to lineage-specific markers for endoderm, ectoderm, and mesoderm. Goat anti-human Otx2, Goat anti-human Brachyury, and Goat anti-human Sox17 were used as antibodies to verify ectoderm, mesoderm, and endoderm differentiation. Also, we used NL557-conjugated donkey anti-Goat (R&D systems, Cat.No.NL001) as the secondary antibody (red). All nuclei were counterstained with DAPI (blue).

RNA isolation and quantitative RT-PCR

Total RNA was isolated using TRIzol and treated with DNase I to remove genomic DNA contamination. According to the manufacturer's instructions, 2μg of total RNA were used for reverse transcription reaction with the RevertAid First Strand cDNA synthesis kit (Fermentas) and oligo (dt) primer. Set up quantitative PCR reactions were performed with the Power SYBR Green Master Mix (Applied Biosystems), and the results were analyzed using a 7500 real-time PCR system (Applied Biosystems). The expression levels of the corresponding genes were normalized to the level of the expression of GAPDH as an internal control compared with the same target gene in HDF. The sequences of the primers are listed in Table 1.

Table1. The sequences of the primers used in this investigation.

Gene	Sequence	Accession Number
GAPDH	CTCATTTCCTGGTATGACAACGA CTTCCTCTTGTGCTCTTGCT	NM_002046.3
Sox17	CGGTATATTACTGCAACTAT GGATTTCCTTAGCTCCTCCA	NM-022454.3
Brachyury	AATCCTCATCCTCAGTTTGG GTCAGAATAGGTTGGAGAATTG	NM_003181.2
Ppar	CTAAAGAGCCTGCGAAAG TGTCTGTCTCCGTCTTCTTG	NM_005037
Islet-1	ATATCAGGTTGTACGGGATCAAATG CACGCATCACGAAGTCGTTC	NM_002202.2
Pax-6	CGGTTTCCTCCTTCACAT ATCATAACTCCGCCCATT	NM_000280.4

Statistical Analysis

Non- parametric Mann-Whitney test using SPSS ver. 16 and P values less than 0.05 were considered significant. Data were presented as the Mean± Standard Deviation (n=3 in each group).

Results

Generation of hiPSCs from Dermal Fibroblasts of Patients with Schizophrenia

Isolation and proliferation of human dermal fibroblasts

Human skin biopsies were cultured in 6- well plates (Figure 2a). Spindle-shaped cells emigrated from the biopsies within 4-5 weeks after culture. Then the biopsies were removed, and the cells were kept for 14 days to grow to approximately 75 percent confluence. The medium was exchanged every other day (Figure 2b).

Characterization of Established hiPSCs

Immunocytochemistry, ALP Test and Karyotype Analysis

After introducing reprogramming factors, cells began to form colonies with hESC-like morphology (Figure 2c), which exhibited intense ALP activity (Figure 2d). The colonies were evaluated by using Immunocytochemistry and by investigating gene expression. They could express pluripotent markers, including Tra-1-60, Oct4, SSEA4, Nanog at the level of protein (Figure 3). The generated putative hiPSCs maintained a normal karyotype (Figure 4).

Differentiation into three germ layers

The generated cells were maintained in a continuous culture by weekly passaging with a split ratio of 1:3. For confirm the multilineage differentiation properties of the cells, They were induced to differentiate into ectoderm, endoderm, and mesoderm. Immunocytochemical staining of differentiated cells showed the expression of Otx2, Brachyury, and Sox-17 at the protein level, as markers of ectoderm, mesoderm, and endoderm; respectively (Figure 5). To evaluate gene expression, Nestin and Pax-6 were evaluated for Ectoderm differentiation; Brachyury and PPAR were studied for Mesoderm differentiation, and Islet-1 and Sox-17 were tested for Endoderm

differentiation. The differentiated hiPSCs could express upregulation of the genes in the derived cells when the results were normalized with fibroblasts (Figure 6).

Discussion

Schizophrenia disease (SCZD) defined as neurodevelopmental disease that makes changes in the process of thoughts, perceptions, and emotions that lead to mental deteriorations. In current study, we generated hiPSCs from dermal fibroblasts of patients suffering from SCZD using episomal vectors in the matrigel coated plates. Our results showed that the derived colonies could express pluripotent markers at the protein level, consist of SSEA-4, Oct-4, Nanog and Tra-1-60. The generated hiPSCs maintained their normal karyotype, and we could also detect strong ALP expression in the colonies. After induction, the cells could express ectodermal (Nestin), endodermal (Islet-1, Sox-17), and mesodermal (Brachyury and PPAR) markers at the level of mRNA when the results were compared with fibroblasts. These data also confirmed the pluripotent properties of the generated cells. Previous reports shown that ips cells can be cultured on mouse embryonic fibroblast as feeders (Ellerstrom, Strehl, et al. 2006; Takahashi, Tanabe, et al. 2007; Crook, Peura, et al. 2007). Alternatively, feeders could be replaced by matrigel, similar to what we have done in our project (Kleinman and Martin 2005). In an investigation, Sun et al. used matrigel as a feeder-free culture condition to

generate individual-specific hiPSCs from an autologous source of cells (Sun, Panetta, et al., 2009).

Moreover, using conditioned media and matrigel supported with essential growth factors could reduce the contamination of stem cells (Ghasemi-Dehkordi, Allahbakhshian-Farsani, et al. 2015). The breaking points for hiPSCs for investigating brain disturbance are neuron and patient variability. This alteration might due to differences in the type of viruses used, epigenetic factors, spontaneous mutation, differences in techniques and take in the cell type of origin. Some scientists have investigated the advantages and difficulties of applying viral integration methods versus episomal or other methods. Viral methods induce genomic deviation (Hussein, Nagy, et al., 2011). They are more efficient than other methods consisting of recombinant protein (Zhou, Wu et al. 2009), miRNA(Miyoshi, Ishii, et al. 2011), and mRNA(Warren, Manos, et al. 2010). Most SCZD patients show different responses to different treatments (Schennach, Meyer, et al. 2012), and only a few percent effectively respond to the therapy (Emsley, Chiliza, et al. 2011). The application of new methods for treating this disease is critical.

Conclusion

The application of hiPSCs derived from schizophrenic patients would be a promising approach for treating the disease and screening the drugs. Further studies are suggested to check the cells' behavior during neurogenesis.

Acknowledgments

The project was pecuniary reinforcement by Iran University (gift number. 96-02-87-31209).

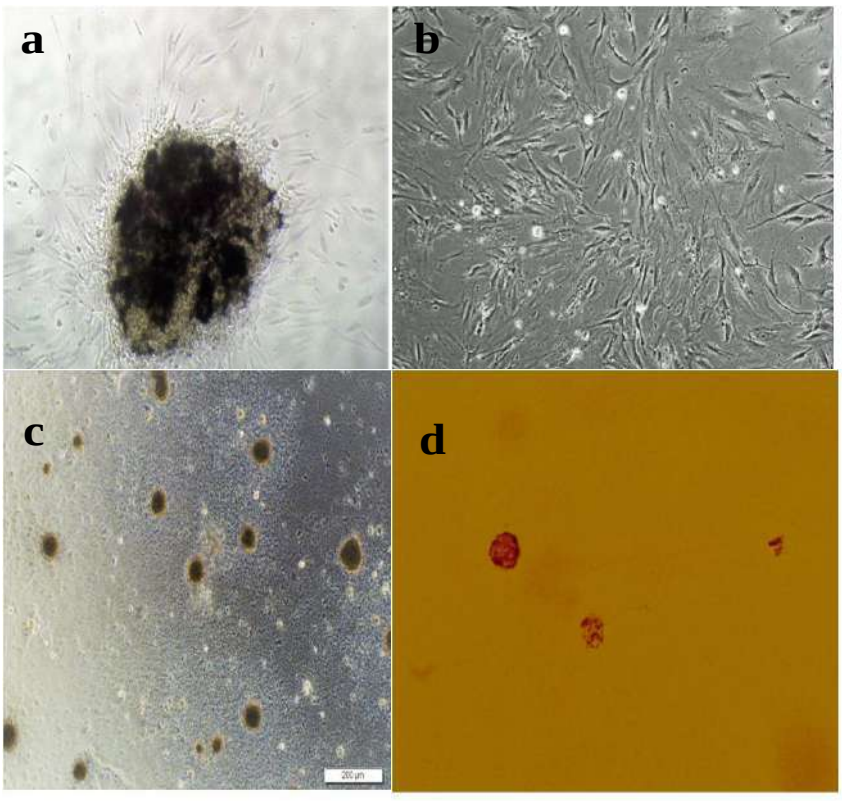

Figure 2. Generation of SCZ patient derived hiPSCs.
(A) Human dermal skin biopsy cultured in a plate at day 14.
(B) SCZ patient derived dermal fibroblast at passage 3.
(C) Human iPSC colonies after induction with episomal vectors.
(**D**) ALP activity test on hiPSC colonies.

Figure2. Generation of Human iPSC- like colonies from SCZ patients. A) Human dermal skin biopsy on day 14. B) SCZ patient-derived dermal fibroblast at passage three. C) Human iPSC colonies after induction with episomal vectors. D) ALP activity test on human iPSC colonies derived from SCZ patients.

Figure3. Characterization of human iPSC-like colonies using immunochemical staining.

The human iPSCs colonies were characterized based on the expression of pluripotency markers, including Oct-4 (a), SSEA-4 (b), Tra-1-60 (c), Nanog (d).

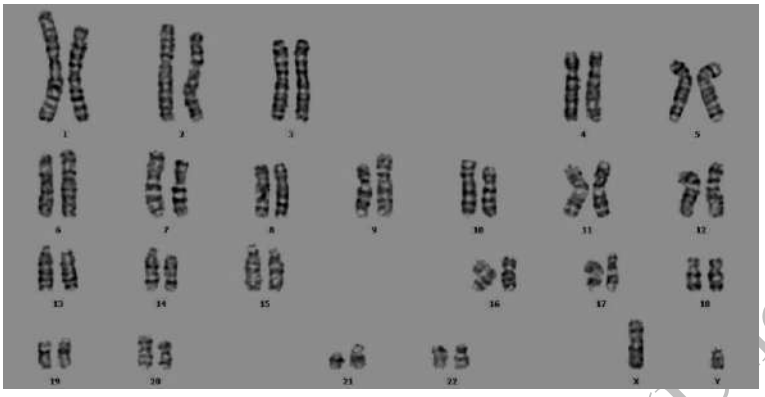

Figure4. Karyotype Analysis.

Generated human iPSCs also maintained a normal karyotype, and cells maintained their normal chromosome morphology.

Figure5. Differentiation of Human iPSC-like colonies

Differentiating human iPSCs colony into mesoderm, ectoderm, endoderm. The cells could express Otx2 (a), Brachyury (b), and Sox-17(c) at the level of protein.

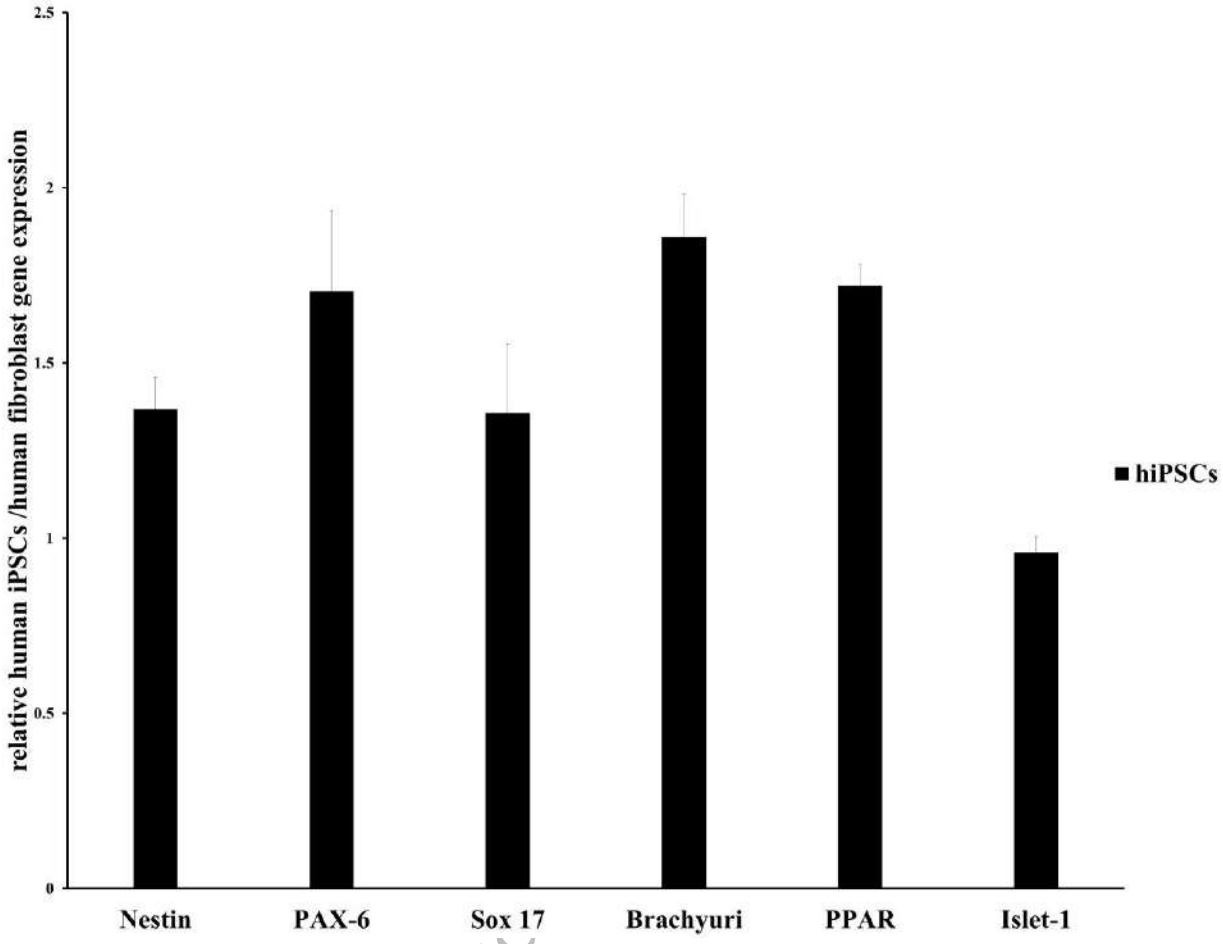

Figure6. Gene expression

Relative Gene expression analysis showed the expression of the ectodermal (Nestin, Pax-6), Mesodermal (Brachyuri, PPAR), and Endodermal (Sox-17, Islet-1) markers in human iPSCs derived from SZDpatients after induction with the corresponding differentiation media. The expression of the same markers in human fibroblast was assumed as the control.

References:

Begre, S. and T. Konig (2008). "Cerebral disconnectivity: an early event in schizophrenia." The Neuroscientist 14(1): 19-45.

Christian, K., H. Song, et al. (2010). Adult neurogenesis as a cellular model to study schizophrenia, Taylor & Francis.

Crook, J. M., T. T. Peura, et al. (2007). "The generation of six clinical-grade human embryonic stem cell lines." Cell stem cell 1(5): 490-494.

Ellerstrom, C., R. Strehl, et al. (2006). "Derivation of a xeno-free human embryonic stem cell line." Stem Cells 24(10): 2170-2176.

Emsley, R., B. Chiliza, et al. (2011). "The concepts of remission and recovery in schizophrenia." Current Opinion in Psychiatry 24(2): 114-121.

Federspiel, A., S. Begre, et al. (2006). "Alterations of white matter connectivity in first-episode schizophrenia." Neurobiology of disease 22(3): 702-709.

Franco, R. and A. Cedazo-Minguez (2014). Successful therapies for Alzheimer's disease: why so many in animal models and none in humans? Front Pharmacol. 2014; 5: 146.

Ghasemi-Dehkordi, P., M. Allahbakhshian-Farsani, et al. (2015). "Effects of feeder layers, culture media, conditional media, growth factors, and passage number on stem cell optimization." Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 85(3): 711-717.

Hussein, S., K. Nagy, et al. (2011). "Human- induced pluripotent stem cells: the past, present, and future." Clinical Pharmacology & Therapeutics 89(5): 741-745.

Jones, C., D. Watson, et al. (2011). "Animal models of schizophrenia." British journal of pharmacology 164(4): 1162-1194.

Kleinman, H. K. and G. R. Martin (2005). Matrigel: basement membrane matrix with biological activity. Seminars in cancer biology, Elsevier.

Maherali, N., R. Sridharan, et al. (2007). "Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution." Cell stem cell 1(1): 55-70.

Marin, O. (2012). "Interneuron dysfunction in psychiatric disorders." Nature Reviews Neuroscience 13(2): 107-120.

Meissner, A., M. Wernig, et al. (2007). "Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells." Nature Biotechnology 25(10): 1177-1181.

Miyoshi, N., H. Ishii, et al. (2011). "Reprogramming of mouse and human cells to pluripotency using mature microRNAs." Cell stem cell 8(6): 633-638.

Palha, J. A. and A. B. Goodman (2006). "Thyroid hormones and retinoids: a possible link between genes and environment in schizophrenia." Brain research reviews 51(1): 61-71.

Rubinov, M. (2013). "Schizophrenia and abnormal brain network hubs." Dialogues in clinical neuroscience 15(3): 339.

Sawa, A. and S. H. Snyder (2002). "Schizophrenia: diverse approaches to a complex disease." Science 296(5568): 692-695.

Schennach, R., S. Meyer, et al. (2012). "Response trajectories in â€œreal-worldâ€ naturalistically treated schizophrenia patients." Schizophrenia Research 139(1-3): 218-224.

Stachowiak, M., A. Kucinski, et al. (2013). "Schizophrenia: a neurodevelopmental disorderâ€"integrative genomic hypothesis and therapeutic implications from a transgenic mouse model." Schizophrenia Research 143(2-3): 367-376.

Sullivan, P. F., K. S. Kendler, et al. (2003). "Schizophrenia is a complex trait: evidence from a meta-analysis of twin studies." Archives of general psychiatry 60(12): 1187-1192.

Sun, N., N. J. Panetta, et al. (2009). "Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells." Proceedings of the National Academy of Sciences 106(37): 15720-15725.

Takahashi, K., K. Tanabe, et al. (2007). "Induction of pluripotent stem cells from adult human fibroblasts by defined factors." cell 131(5): 861-872.

Thomsen, M. S., H. H. Hansen, et al. (2010). "Cognitive improvement by activation of α7- nicotinic acetylcholine receptors: from animal models to human pathophysiology." Current pharmaceutical design 16(3): 323-343.

Tsuang, M. T., W. S. Stone, et al. (2001). "Genes, environment and schizophrenia." The British Journal of Psychiatry 178(S40): s18-s24.

Warren, L., P. D. Manos, et al. (2010). "Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA." Cell stem cell 7(5): 618-630.

Weinberger, D. R. (1987). "Implications of normal brain development for the pathogenesis of schizophrenia." Archives of general psychiatry 44(7): 660-669.

Wernig, M., A. Meissner, et al. (2007). "In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state." nature 448(7151): 318-324.

Yu, J., K. Hu, et al. (2009). "Human- induced pluripotent stem cells free of vector and transgene sequences." Science 324(5928): 797-801.

Yu, J., M. A. Vodyanik, et al. (2007). "Induced pluripotent stem cell lines derived from human somatic cells." Science 318(5858): 1917-1920.

Zhou, H., S. Wu, et al. (2009). "Generation of induced pluripotent stem cells using recombinant proteins." Cell stem cell 4(5): 381-384.