



# Schizophrenia; Recent Cognitive and Treatment Approaches Using Induced Pluripotent Stem Cells

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## Abstract

One of the most severe mental disorder which leads to a disturbance in the percipience of reality is named schizophrenia. Schizophrenia is a combination of hallucinations, delusions, and mental disorders that the severity of them impairs healthy thinking and behavior, and in general, the inability to perform daily activities. Behavioral, intellectual, and emotional disorders indicate the widespread impact of schizophrenia on various aspects of mental health. Schizophrenia's signs and symptoms of schizophrenia are varied, although delusions, hallucinations, or disorganized speech included in common symptoms. Dopamine has been the main subject of much research on schizophrenia for decades. Molecular neuroimaging studies to explore the dopamine system (DA system) in vivo have revealed that Schizophrenia is first associated with a defect in the striatum and then with a defect in extrastriatal regions with centralizing on cortex and midbrain. Also, the gamma-aminobutyric acid (GABA) mRNA dysregulation and neuroinflammatory mechanisms can be useful in schizophrenia. Various medications such as antipsychotic drugs used to the aim of treating this disease, but they can just decline or improve the positive symptoms. Modeling neurodevelopment and synaptic connection defects by induced human pluripotent stem cells have made appropriate circumstances to eliminate schizophrenia treatment barriers. With the development of cell therapies and induced pluripotent stem cells (iPSCs), there is a hope that the negative symptoms can be improved.

**Keywords:** Schizophrenia; iPS cells; iPSCs; GABA; DA hypothesis.

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## Introduction

One of the most severe mental disorders leading to a disturbance in the percipience of reality is named schizophrenia<sup>1</sup>. Schizophrenia has defined by one or all of these following symptoms: delusions, hallucinations, disorganized thinking (speech), and negative symptoms that the severity of them impairs normal thinking and behavior, and in general, disrupts the ability to perform daily activities.<sup>2</sup> Having Schizophrenia requires the patient to receive medication for a lifetime,<sup>3</sup> which imposes a substantial economic burden on the patient and their relatives.<sup>4</sup> Starting treatment in the early stages of the disease is more effective in controlling the progression and preventing more severe symptoms.<sup>1</sup> The onset of schizophrenia in the 20s-30s is common, and the risk for more aged people is remarkably rare.<sup>5</sup> Global statistics investigations revealed that approximately 25 million people are affected.<sup>6</sup> Based on this information and estimation of the economic burden for schizophrenia, the vacancy of more economical and practical therapeutic approaches has felt. To achieving this purpose, precise cognition of symptoms and their causes has required.

## Symptoms

Behavioral, intellectual, and emotional disorders indicate the widespread impact of schizophrenia on various aspects of mental health. Although the signs and symptoms of schizophrenia are very varied, delusions, hallucinations, or disorganized speech included in common symptoms.<sup>7</sup> Based on the general symptom classification, there is three dominant class of schizophrenia symptoms which are including (1) positive (2) negative and cognitive, which have described in detail below.<sup>8</sup>

## Delusions

It is a definite symptom that means false thoughts that are not objective and real. For instance: believing that you have been harassed or harmed. You find yourself under the command of something unrealistic. You are incredibly talented and famous, someone loves you infinitely, announcing a close catastrophe.<sup>9</sup> Almost all people with schizophrenia access delusions. Many recent studies reported an evidenced correlation between delusion and bias against disconfirmatory evidence.<sup>10</sup>

### Hallucinations

It is another positive symptom class and comes when a person's perception feels beyond reality, although a person with schizophrenia experiences these feelings objectively. Hallucinations may occur for all five senses; hearing imaginary noises is more common than others.<sup>11</sup>

### Disorganized Thinking (Speech)

Disorganized thinking has inferred from disorganized speech. It will be impossible to communicate effectively with a person with schizophrenia due to unrelated answers to questions, whether partial or general. On rare occasions, the speech has included expressing sentences that contain meaningless and irrelevant words known as word salad; therefore, it will be incomprehensible to the listener.<sup>12</sup>

### Negative Symptoms

This means that daily activities have almost disrupted. For example, the person may neglect personal hygiene or appear to lack emotion (does not make eye contact, does not change facial expressions or speaks in a monotone).<sup>13,14</sup> Also, the person may lose interest in everyday activities, socially withdraw, or cannot experience pleasure.<sup>15</sup> Periodic improvement and worsening of symptoms have created a wide range of severity and type of symptoms.<sup>16</sup> The onset of symptoms of schizophrenia in men occurs in the middle of the third decade of life, while in women, the symptoms occur in the late twenties.<sup>17</sup>

### Schizophrenia Etiology

Schizophrenia is a complex disease of unknown etiology. The occurrence of schizophrenia as a multifactorial disease is related to genetics, environment, and pathophysiological factors, it shows a combination of behavioral and mental problems ultimately.<sup>18</sup> Schizophrenia is a testament to the intense interaction between genetics<sup>19</sup> and the environment.<sup>18</sup> The neurochemical hypothesis,<sup>18</sup> genetic susceptibility,<sup>20</sup> immunological alteration,<sup>21</sup> and environmental factors such as multiple infections are the primary etiological response of schizophrenia.<sup>22</sup>

### Dopamine Hypothesis

The dopamine hypothesis is the earliest etiology of schizophrenia and has been an important topic of schizophrenia investigations for decades.<sup>23</sup> It has shown that the amount of dopamine in a stratum is more than average in schizophrenia patients compared with healthy people.<sup>24</sup> Excess dopamine intensifies of psychotic symptoms in patients is correlated with increases in released dopamine levels. Excess secretion of dopamine is observant significantly throughout exacerbation in comparison to remission periods.<sup>25</sup> Additionally, using a higher-resolution scanner and more sophisticated region-of-interest analysis methods to identify the striatal

substructures demonstrates that excess striatal dopamine was most prominent in the rostral caudate.<sup>26</sup> Significant enhancement in dopamine secretion throughout the cortex is measurable. Dopamine release in the dorsolateral prefrontal cortex (DLPFC) was significantly positively associated with working memory-related BOLD activation, suggesting a relationship between the blunted release and deficits of frontal cortical function.<sup>27</sup>

### The Role of GABA in Schizophrenia

Some evidence demonstrates the altering of GABAergic neurotransmission in schizophrenia and counted as the main convincing reason for cognitive defects in patients. Postmortem data suggest that GAD67 mRNA translation in the DLPFC has initially decreased through layers 1 to 5, resulting in decreased GAD67 protein levels.<sup>28</sup> It can cause a reduction in the number of neurons in schizophrenia. Based on these studies, it has claimed that the disturbance in GAD67 gene expression occurs exclusively in a specific subset of GABAergic neurons.<sup>29</sup> Additionally, Postsynaptic GABA Receptors can play a crucial role in schizophrenia. Owing to recent advances in medical technology, more accurate investigations of deficits of GABA-A receptor subunits are possible.<sup>30</sup>

### Neuroinflammatory Mechanisms in Schizophrenia

For many years, the association of dopamine levels with schizophrenia has been a significant target of researches. However, these days there is an innovative trend based on new evidence that has concentrated on a particular emphasis on the accurate performance of the immune system.<sup>31</sup> Based on it, the role of neuroinflammation and defect in immune system function is a potent factor in the development of schizophrenia and the symptoms of psychosis in patients.<sup>32</sup> Epidemiological data and historical observations have drawn the attention of researchers to inflammatory processes for decades. Recently extensive research on exposure to infection, stress-induced inflammatory response, glial cell signaling, structural and functional brain changes, and therapeutic trials have confirmed that inflammation can be an effective factor in the appearance and development of schizophrenia.<sup>33</sup> Accordingly, severe infections and autoimmune diseases can increase the risk of schizophrenia and related disorders. Due to aberrations in the immune system and high level of pro-inflammatory cytokines during pregnancy, the mother will be more vulnerable to infections.<sup>34</sup> In seasons when the influenza epidemic is more prevalent, the exposure to infection naturally increases, resulting in an increased risk of congenital disabilities in children prone to schizophrenia.<sup>35</sup> Also, respiratory and reproductive tract infections have considered as an increasing factor of the onset risk of offspring to schizophrenia.<sup>36</sup> Other pathogens linked to schizophrenia include maternal infection with *Toxoplasma gondii*, rubella, measles, polio,

and herpes simplex type 2.<sup>37</sup> Besides studies that have corroborated the role of environmental factors in the occurrence of prenatal infections, attention has focused on the whole genomic studies in new approaches since immune-related genes have discovered in schizophrenia patients.<sup>38</sup> According to this fresh information, recent studies have attempted to identify polymorphisms and immune system alteration to approve the risk of infections that can be rooted in a person's genetics.<sup>39</sup> In addition to environmental factors that cause infection, researches have shown that genetic polymorphisms can increase a person's susceptibility to schizophrenia.<sup>40</sup> These genetic variations can include the immune system divergent genes involved or alterations in response to infectious agents.<sup>41</sup> Extensive study groups have tracked the polymorphisms vulnerable to schizophrenia on different gene loci located on chromosome 6p22. HLA complex genes, moderator the inflammatory response, were the major correlated genes.<sup>42</sup>

### Effective Genes in Schizophrenia

Schizophrenia is a highly heritable disorder with monozygotic concordance rates of approximately 50% and dizygotic concordance rates of approximately 10%. Genomewide association studies have identified several common variants that are associated with schizophrenia.<sup>43</sup> Some of the pathways implicated in these studies have involved neural differentiation, synaptic transmission, and circuit development, lending weight to the hypothesis that schizophrenia is a developmental disorder. There are almost 608 genes that can cause schizophrenia.<sup>44</sup> Principally genes schizophrenia expression should be traceable in organs such as the cerebellum, cerebral cortex, medulla oblongata, thalamus, and hypothalamus.<sup>45</sup> Psychiatric behavioral disorders incorporating schizophrenia genes included ADHD, bipolar disorder, autism spectrum disorder, alcohol dependence, cancer, Alzheimer's and Parkinson's disease, sleep disturbances, and inflammation.<sup>46</sup> By taking advantage of recent studies and approaches, by exploring biological pathways and analyzing genes related to schizophrenia, numerous related genes have identified which listed in Table 1.<sup>45,47</sup>

Biological mechanisms involving synaptic transmission, regulation of membrane potential, and

transmembrane ion transport have identified as before cellular applications related to schizophrenia genes.<sup>48</sup> Additionally, some genetically neurological disorders that their induced pluripotent stem cells (iPSCs) associate with schizophrenia patients. For instance, it has shown in a study that iPSCs with del 22q11.2 (DiGeorge syndrome) identified in schizophrenia patients.<sup>49,50</sup> Furthermore, the variety in copy number of 15q11.2 links to schizophrenia, and it causes a reduction in adherence junctions and retains proper apical polarity.<sup>51,52</sup>

### The Treatments of Schizophrenia

There have been various ways for curing schizophrenia of past decades. Contrary to different functional mechanisms of three classes of antipsychotic medications (conventional typical, atypical, and dopamine partial agonist) operate base on the dopamine system. Additionally, the antioxidant drugs are beneficial during treatment, and cannabidiol has neuronal and molecular effects on the mesolimbic dopamine system.<sup>53</sup> The use of these drugs is not very satisfactory for reasons such as multiple side effects, lack of effect on the negative or cognitive symptom, and not valid for all patients.<sup>54</sup> There are currently no FDA-approved medications available to reduce the negative and cognitive symptoms of schizophrenia, which are the leading causes of patient disruption life.<sup>55</sup> Negative symptoms, such as blunted affect, and social avoidance, and cognitive symptoms, like deficits in attention and executive function, reduce capable of life, extremely.<sup>56</sup> Even among schizophrenia patients who respond to antipsychotic medications, disability is significant, and functional consequences are disappointing. Therefore, the need for immediate treatment for negative and cognitive symptoms of schizophrenia is greatly felt.<sup>57</sup> The complexity and multifactorial nature is a major obstacle to an accurate knowledge of schizophrenia, and as a result, it has become almost impossible to find a completely effective treatment for this disorder.<sup>58</sup> Human-induced pluripotent stem cells (hiPSCs) supply a high-potential domain for a more accurate study of neurological disorders like schizophrenia. HiPSC provides an adequate opportunity that permits researchers to model and studies the exact mechanisms of the schizophrenia progression.<sup>59</sup>

### The Induced Pluripotent Stem Cells

In a groundbreaking discovery, Yamanaka showed that ectopic overexpression of the four transcription factors c-Myc, Klf4, Oct4, and Sox2 could reprogram fibroblasts into iPSCs.<sup>60</sup> The human iPSCs generated in this manner share many characteristics with human blastocyst-derived embryonic stem cells (hESCs), including indefinite self-renewal, clonogenicity, and pluripotency (the ability to differentiate into all adult cell types in the body).<sup>61</sup> Hence, this reprogramming process enabled the generation of

**Table 1.** Gene Families Involved in Schizophrenia

Gene Categories	Genes Name
Glutamnergic receptors	<i>GRIA1, GRIN2, GRIK4, GRM5</i>
Serotonergic receptors	<i>HTR2A, HTR2C</i>
GABAergic receptors	<i>GABRA1, GABRB2</i>
Dopaminergic receptors	<i>DRD1, DRD2</i>
Calcium-related channels	<i>CACNA1H, CACNA1B</i>
Solute transporters	<i>SLC1A1, SLC6A2</i>
Neurodevelopmental genes	<i>ADCY1, MEF2C, NOTCH2, SHANK3</i>

hESC-equivalent pluripotent stem cells in the laboratory from readily available adult somatic cells, without any need for a human blastocyst.<sup>62</sup> Human iPSCs can be differentiated into neurons using a variety of methods. Initial studies of schizophrenia using iPSCs have yielded some promising results.<sup>63</sup> The first such study showed that neurons from schizophrenia patients had regular functional properties (as measured with electrophysiology and Ca<sup>2+</sup> imaging), but displayed reduced connectivity, neurites, and synaptic protein levels.<sup>64</sup> Schizophrenia neurons also had altered expression of genes involved in the Wnt, cAMP, and glutamate-signaling pathways.<sup>65</sup> A later study of these iPSCs showed that schizophrenia neural progenitor cells (NPCs) had an abnormal expression of genes and proteins related to cytoskeletal remodeling and oxidative stress pathways.<sup>66</sup>

### *Appropriate Cells in the Brain to Modeling the iPSC-Derived Cells*

There is no enough evidence to identify the relevant type of iPSCs-derived cells that can appear related symptoms to the disease. Although postmortem brains from patients demonstrate a decline in the volume of the cortex.<sup>67</sup> Furthermore, it has shown that the volume of the hippocampus has reduced in patient's brains. Especially in the dentate gyrus and cornu ammonis 3 hippocampal fields.<sup>68</sup> Also, schizophrenia patients, the pyramidal neurons in the cortex, the spine density, and synapse numbers are decreased.<sup>69</sup> As a result, the cell models from the cortex and hippocampus of schizophrenia patients can be appropriate to show the schizophrenia disease features and identify the new treatment approaches. The cortical neurons and hippocampal dentate gyrus neurons can use as differentiated cells from human iPSCs. The rate of homogeneity is noticeable in cultures.<sup>70</sup> The heterogeneous neuronal cultures are beneficial since they are more similar to their original niches in the brain.

### *iPSC-Derived Cells Approach*

To achieve advanced and effective treatment for schizophrenia by using stem cell-derived neurons; generally, two approaches are studied.

### *Reprogramming and Inducing Cells Using Viruses*

For the first time, Chiang et al. claimed that hiPSCs have derived from schizophrenia patients; indeed, there was no information about differentiation.<sup>71</sup> Brennard et al presented a new founded based on a defect of SCZD hiPSCs in neuronal connectivity. Additionally, they reported a significant reduction of post syntactical gene expression.<sup>59</sup> In the first step, the iPSCs should reprogram. So the primary human fibroblasts are reprogrammed using inducible lentiviruses.<sup>72</sup> There are two fundamental limitations to using viruses to reprogramming cells. First of all, the lack of complete suppression of viral genes, and

the second is the risk of viral mutagenic gene insertion.<sup>59</sup> So, recently a doxycycline-inducible lentiviral system applied to silence viral genes completely.

While using adenovirus, plasmid vectors, however, with low efficiency, and synthetic modified mRNA approaches are developing for reprogramming.<sup>73</sup> In the first step, and inducible lentiviruses are engaged to reprogram the primary human fibroblast. Schizophrenia hiPSCs have no evident deficiency in differentiation NPCs and neurons.<sup>74</sup> To ensure the differentiation of hiPSC to the NPCs and neurons, the trans-neuronal spread of rabies is a reliable tool for evaluating synaptic communication. The synaptic contacts make rabies transmission and are associated with synaptic input potential.<sup>75</sup> Though there are ambiguous sides of rabies trans-neuronal tracing, NCAM as a presynaptic protein indicates reduced expression in schizophrenia hiPSC neurons.<sup>59</sup> Trans-neural detection is the result of reduced neural connections; it does not necessarily lead to reduced synaptic function, in schizophrenia hiPSC neurons. In postmortem human studies and animal models repeating of some cellular and molecular phenotypes in hiPSC neurons from heterogeneous patients with similar deficits perceived. The decreased in neuronal connectivity in schizophrenia hiPSC neurons was observed.<sup>76</sup> Expression studies of hiPSC neurons provide this possibility of intuition of antipsychotic treatment on live, genetically identical neurons with an effective response to treatment and premitting of confounding variables of postmortem analysis such as treatment history, drug or alcohol abuse, and cause of death.<sup>77</sup> In the schizophrenia hiPSC neurons, of the 596 unique genes differentially expresses and the hypotheses based on pharmacological and genomewide association (GWAS) studies of schizophrenia has confirmed by gene expression profiles of schizophrenia hiPSC neurons and additionally, it identifies some novel pathways including NOTCH signaling, SLIT/ROBO axon guidance, EFNA mediated axon growth, cell adhesion, and transcriptional silencing.<sup>59</sup> Multiple genes involved in increasing schizophrenia hiPSC neurons efficiency depend on schizophrenic pathways and have not recognized as specific genes involved in this disease, such as PDE family genes, ADCY8, and PDE4B.<sup>78</sup> The use of human iPSCs to model individual patient's cells has reinvigorated the hope that a new wave of targeted treatment opportunities will emerge soon. The five antipsychotic drugs have tested to modify neuronal connectivity in vitro. In the last 21 days of neuronal differentiation, clozapine, loxapine, olanzapine, risperidone, and thioridazine have distributed. The loxapine in all patients is effective in enhancing the connection between neurons<sup>77</sup> and realizing the best possible concentration, and the best time to administer drugs upgrades the antipsychotic medication efficiency.



### Interneuron Transplants, Derived From Embryonic Stem Cells

It has demonstrated that within developmental processes, the parvalbumin (PV) somatostatin (SST)-positive interneurons are branched from the medial ganglionic eminence. The fetal medial ganglionic eminence tissue has used to reconstitute the function of interneurons in the methyl azoxy methanol (MAM) developmental model of schizophrenia.<sup>79</sup> The interneuron transplants into the ventral hippocampus decrease the hyperactivity of hippocampal and also dopamine operation, and the amphetamine-induced locomotor activity will be at a regular rate that is a model for positive symptoms of schizophrenia.<sup>80</sup> By this method, the positive symptoms may be cured in the MAM model of schizophrenia and can be a functional approach to improving the negative and cognitive symptoms of patients whose treatment was not successful with antipsychotic medications.<sup>59</sup>

The observations show that the SST- and PV-enriched transplants can modify the behavioral disease differently. The transplants of SST and PV both can change the ventral hippocampus hyperactivity.

Unique anatomical, biochemical, and physiological characteristics of SST and PV make this variety of effects. For instance, the firing pattern can specialize in the PV- and SST-positive interneurons. As shown, the dopamine hypothesis claims that increased dopamine levels in the mesolimbic stimuli the positive symptoms, but by this method, both SST- and PV-positive interneuron transplants into the ventral hippocampus decline the dopamine population activity in MAM-treated animal models.<sup>78</sup>

One of the cognitive flexibilities is reversal learning, which shows a defect in schizophrenia, and reversal-learning has associated with orbitofrontal activity and subcortical dopamine function. Also, community relations and extra-dimensional set-shifting, which linked with a part of the brain that receives direct projections from the ventral hippocampus and in schizophrenia patients are not regulated well, can be improved with only PV-positive transplants and can improve the disorders.<sup>81</sup> Generally, this strategy could be useful for the treatment of schizophrenia. However, the timing of the transplant should consider. The cells should be transplanted between postnatal day (PND) 40–45 and circulating androgen dosage. For using the MAM rodent model, the cell transplantation must perform as early as PND 40.<sup>76</sup> Another practical factor that should consider is the stage of schizophrenia. So the transplantation will be most effective if done in the earliest stage of the disease.

### Conclusion

Brain-imaging studies were the first approach in schizophrenia investigations. Although structural differences in healthy and patient groups have observed,

brain studies were unable to determine the damaged parts of the nervous system accurately.<sup>82</sup> accurately.

On the other hand, it identified the effective pattern of dopamine and glutamate in schizophrenia and applied the pharmacological treatment to improve severe symptoms. GWAS studies, with the onset of the genetic age, is beginning. These studies presented documented reports based on susceptibility to infection of polymorphisms.<sup>83</sup> Nevertheless, recent advances in cell therapy have given researchers an unprecedented and immense circumstance for recognizing and treating schizophrenia. While pharmacological treatment exclusively attempts to attack schizophrenia symptoms, cell therapy seeks to improve other neurological diseases, including Parkinson's and stroke. The capacity of cell transplants in schizophrenia is not known well yet. Therefore there are lots of features of this that should achieve.

### Conflict of Interest

The authors declare that they have no conflict of interests.

### Ethical Statement

Not applicable.

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