Genetic Expression of Metabotropic Glutamate Receptor 7 in Schizophrenic Patients Using Human Induced Pluripotent Stem Cells

Dalia Khalifa Zayet, Mahmoud Batrawy, Heba Fathy Eid, Hoda Abdou Hussein

Corresponding Author: Dalia Khalifa Zayet
daliakhalifa_z@yahoo.com

ABSTRACT

Background: The ability of metabotropic glutamate receptors to modulate the neurotransmission of glutamate has attracted great attention for its role in the pathophysiology of schizophrenia and also for its potential ability to be a target for the development of novel antipsychotics. Metabotropic glutamate receptor 7 has a wide expression in the cortex, hippocampus, and other forebrain regions. It has an important role in short-term plasticity and multiple learning processes. Exploring the molecular changes and cellular genetic expression of metabotropic glutamate receptor 7 gene is an essential step to know more about its real functioning in patients presenting with schizophrenia. Aim: Measuring the genetic expression of metabotropic glutamate receptor 7 in patients presenting with schizophrenia and comparing it in healthy controls. Methods: Modeling of schizophrenia through human induced pluripotent stem cells (hIPSc) through reprogramming of fibroblasts into pluripotent stem cells followed by neuronal differentiation. Real time Polymerase chain reaction was used for measurement of genetic expression. Results: There were lower means of metabotropic glutamate receptor 7 gene expression in the patients group (0.843) than the control group (1.051) but with no statistically significant difference between the 2 groups (P=0.241). Conclusion: There was no significant alteration of the genetic expression of metabotropic glutamate receptor in schizophrenic patients. However, further studies are needed with larger samples to verify its role in pathophysiology of schizophrenia.

Keywords: Genetic, Expression, Glutamate Receptor 7, Schizophrenia, Stem Cells

1. INTRODUCTION

According to the WHO, 15% of human diseases will be associated with mental disorders by the year 2020 which will lead to higher social and personal burden. One of the most important mental illnesses that gained the attention of neuroscientists in the past three decades is schizophrenia. Understanding more about the etiology of this disorder may lead to earlier intervention and consequently better prognosis(1).

Many studies have focused on the genetic expression of glutamate as a part of the underlying etiology in schizophrenia due to its important role in the developmental processes such as synaptogenesis, cellular migration, cell signaling and mitochondrial functioning which are implicated in the pathogenesis of many neurodevelopmental disorders(2).

The glutamatergic receptors are formed of two types which are ionotrophic and metabotropic glutamate receptors. The metabotropic receptors are further classified into three groups with eight subtypes. They have important role in
modulation of glutamate release and N-methyl-D-aspartate (NMDA) receptor functioning making them a target for novel antipsychotics. Metabotropic glutamate receptor 7 (mGLUR7) is one of the important subtypes which constitute the widest expression of group III metabotropic receptors especially in the cortex and hippocampus. Studies on mice have proved its role in hippocampal short term plasticity, working memory and learning process. In schizophrenia, polymorphism in the gene encoding mGLUR7 was found in a large Japanese cohort study. Also, another study found that the activation of mGLUR7 decreases thalamocortical activation which is thought to be an overactive circuit in schizophrenia. However, still few studies have discussed the potential relation between altered expression of metabotropic glutamate receptor 7 and schizophrenia.

In addition, there were many obstacles in investigating genetic expression of neurotransmitters in the human living brain. Some studies used blood sampling which aroused a question mark about its real representation of the neural tissue especially in the field of genetic expression and functioning. Other studies used postmortem samples but this faced lots of problems that included difficulty in obtaining brain samples and absence of correlations with any clinical findings. Moreover, it revealed little about disease initiation and progression. Animal models remained the solution for these obstacles for years. However, in animal models, there are species-specific differences between the human and animal brain and the lack of translatable animal models, particularly for the disorders in which higher function is involved. In order to overcome these limitations, the generation of a new study model was necessary for developing the knowledge of the neurobiology of human psychiatric diseases.

This was achieved through modeling of neuropsychiatric disorders through human induced pluripotent stem cells (hIPSc) which depended on reprogramming of somatic cells into hIPSc then differentiating it into human neurons and glial cells. This methodology is hoped to be a transformative step in the field of research which would help us to understand more about genetic functioning, molecular and cellular pathophysiology in neuropsychiatric disorders.

So in this study, we aimed to measure and detect differences in the genetic expression of metabotropic glutamate receptor 7 (mGLUR7) in patients presenting with schizophrenia and comparing to that in healthy controls using human induced pluripotent stem cells reprogramming technology.

2. SUBJECTS AND METHODS

This study is a cross sectional study. The aim in this study was to investigate the genetic expression of metabotropic glutamate receptor 7 (mGLUR7) genes in patients and controls. The genetic expression was measured on patient specific neurons that were obtained from reprogramming of fibroblasts into human induced pluripotent stem cells then differentiation into nerve cells.

The sample in this study consisted of 24 participants: 11 patients presenting with schizophrenia and 13 matched control in age and sex. The case study was conducted on patients presenting with schizophrenia from the outpatient clinic and the inpatient ward of the department of psychiatry. They were all fulfilling Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision criteria of Schizophrenia. As for control group, they were selected from the plastic surgery department.

Exclusion criteria for the patients included history of head trauma, current mood disorder, history of electroconvulsive therapy sessions in the past six months and dermatological disorders such as melanoma or nevus to avoid distorted fibroblasts. As for the control group, the exclusion criteria included past history of psychiatric disorder, history of head trauma, history of dermatological disorders.

The cases were subjected to Semi-structured interview: It was applied by using a modified clinical sheet of psychiatry department designed to diagnose different psychiatric disorders according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision criteria. This clinical sheet starts by covering all the basic demographic data followed by the complaint reported by the patient and the informant, history of presenting illness, past psychiatric history, substance history, family history, present mental state, risk assessment diagnosis and current treatment. Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision for diagnosis of psychotic disorder was also applied. All participants were assessed for psychiatric symptoms using the Positive and Negative Syndrome Scale for Schizophrenia.

The first step was taking a punch skin biopsy from the patients after taking the patients' first degree relative consent and the patients' assent. As for the control

group, the skin samples were taken from the patients that underwent plastic surgeries such as abdominoplasty, skin grafts and breast reduction after taking their consents. In these operations, excision of healthy skin is an essential step. The control group was selected from the plastic surgery department. The participants fulfilled the inclusion criteria.

The next step was reprogramming of fibroblasts into human induced pluripotent stem cells via reprogramming System which uses vectors based on replication-incompetent Sendai virus to safely and effectively deliver and express the necessary key genetic factors. In contrast to many available protocols, which rely on viral vectors that integrate into the genome of the host cell, this reprogramming system used vectors that are non-integrating and remain in the cytoplasm (i.e., they are zero-footprint).

In addition, the host cell were cleared of the vectors and reprogramming factor genes by exploiting the cytoplasmic nature of Sendai virus and the functional temperature sensitivity mutations introduced into the key viral proteins.

Differentiation of human induced pluripotent stem cells into neurons occurred through generation of Neural Progenitor Cells occurred using using Collagenase (1 mg/ml in DMEM) for digestion at 37°C for one to two hours until colonies lifted from the plate then the next step was differentiation of neurons into GABergic neurons, glutamatergic neurons, and dopaminergic neurons via were dissociated with Accutase (1 unit/ml, Invitrogen) at 37°C for 5 min and placed onto polyornithine/laminin-coated coverslips at day 26 in Neurobasal medium in the presence of valproic acid (VPA, 10 μM, Sigma) for 1 week, followed by a set of trophic factors, including brain-derived neurotrophic factor 20 ng/ml, glial-derived neurotrophic factor (GDNF, 10 ng/ml), and cyclic adenosine monophosphate cAMP (1 μM). Real time polymerase chain reaction was used to assess the genetic expression of metabotropic glutamate receptor 7 in the neurons generated from patients and controls.

The study took the approval of the ethical committee. Informed written consents were taken from the patients first degree relatives and from the controls. Informed assent were taken from the patients presenting with schizophrenia before performing any procedure.

### Statistical analysis

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Mann Whitney U test for independent samples. For comparing categorical data, Chi square (χ²) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between various variables was done using Spearman rank correlation equation. p values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows.

### 3. RESULTS

In the patients group, the age ranged from 18 to 45. The sample of patients included thirteen males and seven females. Onset of psychosis ranged from one year to 22 years with mean approximately 9 years. Patients were taking different types of antipsychotics such as risperidone, clozapine, trifluoperazine and aripiprazole. The mean of positive symptom on PANSS scale is 24.5 and the mean of negative symptoms is 21.4 (minimum score 0, maximum score 42). As for general psychopathology, mean was 42.5 (minimum score 0, maximum score 112).

There was lower means of m GLUR7 gene expression in the patients group (0.843) than the normal control group (1.051) with no statistical significant difference between the 2 groups (P=0.246) as shown in figure (1).

![Figure 1: Metabotropic glutamate receptor 7 gene (mGLUR7) expression mean values in patients and normal control groups](image-url)
There was upregulation (fold change $\geq 1$) of mGLUR7 in 8 patients out of 11 patients representing 73% of generated neurons in the patient group. There was upregulation of mGLUR7 in 9 patients out of 13 participants representing 70% of generated neurons in the patient group as shown in the table 1 and 2.

### Table 1: Pattern of Metabotropic glutamate receptor 7 gene expression in patients group

<table>
<thead>
<tr>
<th>Patients</th>
<th>Primary reaction</th>
<th>End reaction</th>
<th>Fold change</th>
<th>Type of regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.85</td>
<td>12.5</td>
<td>1.29</td>
<td>Upregulation</td>
</tr>
<tr>
<td>2</td>
<td>4.2</td>
<td>12</td>
<td>1.2</td>
<td>Upregulation</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>12.8</td>
<td>1.31</td>
<td>Upregulation</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>13.2</td>
<td>1.2</td>
<td>Upregulation</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>1.2</td>
<td>0.13</td>
<td>Downregulation</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>14</td>
<td>1.4</td>
<td>Upregulation</td>
</tr>
<tr>
<td>7</td>
<td>4.5</td>
<td>12.85</td>
<td>0.83</td>
<td>Downregulation</td>
</tr>
<tr>
<td>8</td>
<td>4.2</td>
<td>13.1</td>
<td>0.16</td>
<td>Downregulation</td>
</tr>
<tr>
<td>9</td>
<td>2.85</td>
<td>1.55</td>
<td>1.16</td>
<td>Upregulation</td>
</tr>
<tr>
<td>10</td>
<td>4.5</td>
<td>15.5</td>
<td>1.16</td>
<td>Upregulation</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>13.5</td>
<td>1.09</td>
<td>Upregulation</td>
</tr>
</tbody>
</table>

### Table 2: Pattern of Metabotropic glutamate receptor 7 gene expression in control group

<table>
<thead>
<tr>
<th>Control</th>
<th>Primary reaction</th>
<th>End reaction</th>
<th>Fold change</th>
<th>Type of regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>10.5</td>
<td>1.42</td>
<td>Upregulation</td>
</tr>
<tr>
<td>2</td>
<td>4.5</td>
<td>10.2</td>
<td>1.08</td>
<td>Upregulation</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>9.95</td>
<td>2.15</td>
<td>Upregulation</td>
</tr>
<tr>
<td>4</td>
<td>4.25</td>
<td>9.95</td>
<td>0.3</td>
<td>Upregulation</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10.5</td>
<td>1.5</td>
<td>Upregulation</td>
</tr>
<tr>
<td>6</td>
<td>5.55</td>
<td>5</td>
<td>0.41</td>
<td>Downregulation</td>
</tr>
<tr>
<td>7</td>
<td>3.85</td>
<td>11.5</td>
<td>1.0</td>
<td>Upregulation</td>
</tr>
<tr>
<td>8</td>
<td>4.25</td>
<td>4</td>
<td>1.23</td>
<td>Downregulation</td>
</tr>
<tr>
<td>9</td>
<td>4.85</td>
<td>9.95</td>
<td>0.94</td>
<td>Upregulation</td>
</tr>
<tr>
<td>10</td>
<td>3.9</td>
<td>10.5</td>
<td>1.06</td>
<td>Upregulation</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>12</td>
<td>1.21</td>
<td>Upregulation</td>
</tr>
<tr>
<td>12</td>
<td>5.5</td>
<td>12</td>
<td>1.07</td>
<td>Upregulation</td>
</tr>
<tr>
<td>13</td>
<td>4.5</td>
<td>11</td>
<td>0.93</td>
<td>Upregulation</td>
</tr>
</tbody>
</table>

### 4. DISCUSSION

Schizophrenia is a chronic and one of the most severe mental disorders. Impaired functioning, poor quality of life, stigma, early mortality are some of the main features and costs of this mysterious disease. Many studies were pointing towards cognitive impairment as the main core of schizophrenia. Knowing more about the cellular, molecular and genetic changes behind this cognitive impairment may be a starting point in the hope of cure. Many important studies were done on genetic polymorphism$^{(10)}$. Less number of studies was done on genetic expression mostly on postmortem brains$^{(11)}$. However, the main aim here was to investigate genetic expression in patients presenting with schizophrenia and correlating it to the cognitive impairment with the help of human induced pluripotent stem cell technology.

The main interest in this study was investigating the expression of the above mentioned genes. The genome is the same in each neuron from the 10 billion neurons of the brain and also the same in all types of cells in the body. So, the way of how a neuron becomes functioning or malfunctioning in a mental disorder depends mainly on which specific genes are expressed and which is silenced. This means that the patient may inherit a risk but what magnify that risk or abolish it is the genetic expression$^{(12)}$.

Till few years ago, genetic expression was not possible in living patients except through blood samples. This always used to arouse a question mark about its real representation of the neural tissue. Recently, in vitro culture of human neurons and glial cells became possible$^{(13)}$, a fact that solved the problem that was present for decades in Studying neuropsychiatric...
disorders in general and schizophrenia in particular which was the difficulty in investigating the site of the lesion directly i.e: the neurons of the affected brains. This triggered our aim to apply this new technology in a sample of Egyptian schizophrenic patients.

As mentioned before, Metabotropic receptors are classified into: Group I metabotropic glutamate receptors (1 and 5), Group II (2 and 3) and III (4 and 6-8). we chose to investigate the mGlu7 receptor subtype, which belongs to the mGlu III group, seems to play a special role in neurodevelopment and in the pathophysiology of schizophrenia\(^\text{14}\).

In this study, there was upregulation of metabotropic glutamate receptors 7 (GRM7) in both patients and control group with no statistically significant difference between the 2 groups (P=0.246). Many studies were done on polymorphism of mGLUR7 and its relation in the pathophysiology of schizophrenia but very limited studies were done on expression of the gene. Its suggested role in cognitive impairment made it an important candidate gene for assessment of genetic expression. A study reported association of schizophrenia with polymorphisms in group III metabotropic glutamate receptor genes, 4 and 7 on 100 case-control pairs of Japanese and identified two neighboring single nucleotide polymorphisms in GRM7 showing highly significant haplotype association with schizophrenia\(^\text{15}\).

Another study done in 2015 confirmed that metabotropic glutamate receptors 7 has been identified as a candidate gene for many psychiatric disorders especially schizophrenia. This study was done on 1034 schizophrenic patients and 1034 healthy controls from China. They investigated whether single nucleotide polymorphisms in GRM7 were associated with schizophrenia. Their results showed that the two single nucleotide polymorphisms demonstrated significant difference between schizophrenic patients and control subjects in allele frequencies indicating that these GRM7 SNPs might be associated with schizophrenia\(^\text{16}\).

In another study, it was reported that Of the eight metabotropic glutamate (mGlu) receptor subtypes, only mGlu7 is expressed presynaptically at the Schaffer collateral -CA1 synapse in the hippocampus in adult animals and it was found that activation of metabotropic Glutamate Receptor 7 is required for induction of long-term potentiation in the hippocampus which denotes its importance in cognition and learning\(^\text{17}\). As for human studies using human induced pluripotent stem cells, still there are very few studies that explore the genetic expression profile in patients with schizophrenia. This is because it is relatively emerging new field of research in psychiatry which gives the chance for unlimited ideas of research. Scientists search in different aspects making data still more or less limited and diffuse. However, it is only a matter of time till considerable data in each field grows up.

In a systematic review done in 2016, publications were reviewed until June 2015. They found only 80 publications in using induced pluripotent stem cell in psychiatry: 48 research articles and 33 reviews. Research in schizophrenia contributed with 33% (approximately 16 articles)\(^\text{18}\). These 16 articles targeted different topics such as mitochondrial changes, genetic expression, synaptic connections, etc. From these limited number of new emerging studies, we found only one study was done in on 4 patients presenting with schizophrenia investigating the gene expression of GABA beta 2 and metabotropic glutamate receptor 7. It did not show significant changes in the expression of the former and showed downregulation of the later\(^\text{19}\). However, the limited number of participants and the absence of controls may explain the absence of significant changes.

5. CONCLUSION

There was no significant alteration of the genetic expression of metabotropic glutamate receptor in schizophrenic patients. However, further studies are needed with larger samples to verify its role in pathophysiology of schizophrenia.

REFERENCES

