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RESEARCH ARTICLE OPEN ACCESS

ROLE OF SEROTONIN TRANSPORTER GENE IN SCHIZOPHRENIA

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ABSTRACT

Schizophrenia is a complex mental disorder due to the involvement of several genes. Schizophrenia is mainly due to the abnormal brain function. This abnormalities is mainly involved in the transferring and processing of information's in the brain. Nerve cells in the brain communicate with each other by releasing chemicals from the nerve endings. These chemicals are called neurotransmitters. Many of the symptoms of Schizophrenia have been linked to the abnormal activity of particular neurotransmitters. Serotonin is a type of neurotransmitter that have a major role in Schizophrenia. Serotonin transporter gene (SLC6A4)is one of the most promising candidate gene in the psychiatric disorder, Schizophrenia. Serotonin transporter gene encodes an integral membrane protein that transports the neurotransmitter serotonin from synaptic spaces into presynaptic neurons. The encoded protein terminates the action of serotonin and recycles in a sodium dependent manner. A single nucleotide polymorphism (SNP) in the promoter of this gene has been shown to affect the rate of serotonin uptake and then cause the abnormal brain function.In the study the investigation is mainly based on the potential influence of the SLC6A4 gene on the psychopathology of Schizophrenia due to the SNPs in SLC6A4 gene. From the data's obtained from sequence analysis, there were clearly observed the SNPs of SLC6A4 gene. These sequence analyzeddata's were then subjected to the statistical analysis for further clarification of the results. Data's obtained from the statistical analysis it is significantly observed the role of serotonin transporter gene in the most distressing and disabling mental disorder,Schizophrenia.

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INTRODUCTION

The first signs of schizophrenia tend to surface in adolescence or young adulthood. People with schizophrenia suffer from problems with their thought processes. This leads to hallucinations, delusions, disordered thinking and unusual speech or behavior. Symptoms affect the ability to interact with others and often people with schizophrenia withdraw from the outside world. Schizophrenia is a complex illness and scientists believe it is caused by a number of different factors that act together .These factors include genetic influence, trauma (injury) to the brain occurring during or around the time of birth, as well as the effects of social isolation and stress. There is no known single cause for Schizophrenia. Not all the factors that play a role in schizophrenia are understood. However, experts agree that the disease is due to abnormal brain function. Schizophrenia is an illness of the brain. Scientists believed that its symptoms are caused by abnormalities in the transfer and processing of information in the brain. Nerve cells in the brain communicate with each other by releasing chemicals from their nerve endings. These chemicals are called neurotransmitters. Many of the symptoms of schizophrenia have been linked to abnormal activity of particular neurotransmitters. The words chizophrenia comes from a Greek word "shjzofre'neja" meaning 'split mind'. About 1% of the world population is affected by schizophrenia. In India, for a population of nearly 1 billion people, an estimate of 4 million people with schizophrenia are present. Generally, men show the first signs of schizophrenia in their mid-20s and women show the first signs in their late 20s. (Coleman et al., 1996). The incidence of schizophrenia among parents, children, and siblings of patients with the disease is 15%. Among identical twins, the tendency to have the same schizophrenic illness is only about 30-50%. This shows that it is not caused entirely by genetic factors. In Kerala, schizophrenia is 1.3% of the burden diseases. Schizophrenia is a complex disorder due to its complexity in symptoms and involvement of several genes. The symptoms of schizophrenia can be divided into two types: Positive and negative. Positive symptoms can be defined as excesses or distortions of normal mental functions and includes hallucinations, delusions, disorganized thinking and agitation. Negative symptoms represent a loss or reduction of normal functioning and are more

difficult to evaluate because they may be influenced by a concurrent depression or a dull and unstimulatory environment.

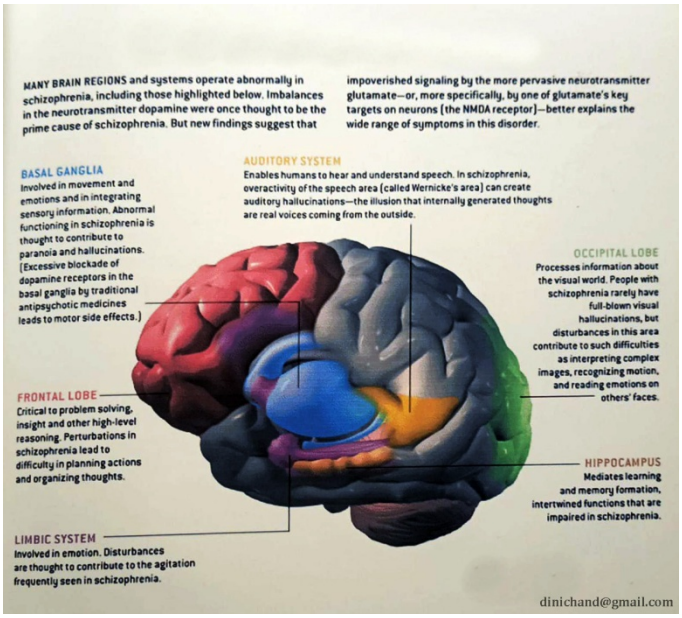

Fig 1. The brain in schizophrenia

Inappropriate emotions. Their disorganized behaviors may disrupt normal activities, such as showering, dressing and preparing meals. Undifferentiated type schizophreniais characterized by some symptoms seen in all the above types but not enough of any one of them to define it as another particular type of schizophrenia. Residual type schizophrenia is characterized by a part history of at least one episode of schizophrenia, but the person currently has no positive symptoms. The treatments for schizophrenia are, Medication - The first line pharmacological therapy for schizophrenia is usually the use of antipsychotic medication (The Royal College of Psychiatrists., 2003). Psychosocial therapy -Psychosocial therapies includes rehabilitation which focuses on social skills and job training to help people with schizophrenia function in the community and live independently. Electro Convulsive Therapy (ECT) - This is a procedure in which electrodes are attached to the person's head and a series of electric shocks are delivered to the brain. The shocks induce seizures, causing the release of neurotransmitters in the brain. The exact cause of schizophrenia is not yet known but the genetic and environmental factors are considered responsible for the same. Functional differences in brain activity are heavily linked to neuro cognitive deficits, which often occur with schizophrenia. There is also evidence that prenatal exposure to infections increases the risk for developing schizophrenia later in life. The serotonin transporter gene (SLC6A4) is a candidate gene for schizophrenia based on serotonin transporter's crucial role in serotonergic neurotransmission. Serotonin transporter gene (SLC6A4 gene-Solute carrier family 6 neurotransmitter transporter, serotonin) is one of the most promising candidate gene for the psychiatric disorder, Schizophrenia. The gene that encodes the serotonin transporter is called solute carrier family 6 neurotransmitter transporter, serotonin. In humans the gene is found on chromosome 17. This gene encodes an integral membrane protein that transports the neurotransmitter serotonin from synaptic spaces into pre synaptic neurons. A single nucleotide repeat length polymorphism in the promoter of this gene has been shown to affect the rate of serotonin uptake and may play a role in abnormal brain functions.

MATERIALS AND METHODS

Blood samples were collected with informed consent from patients suffering from schizophrenia.DNA isolation was performed using standard organic extraction methods.PCR was performed using specific primers.

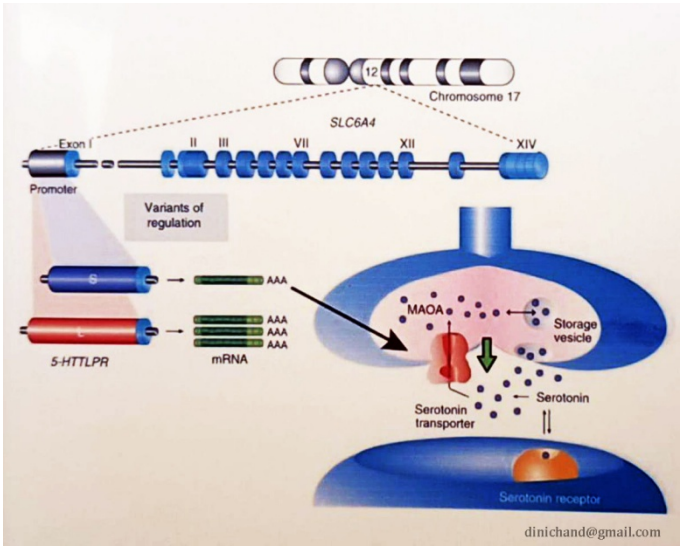

Fig 2 – Mechanism of slc6a4 gene in processing of serotonin neurotransmitter in brain

Agarose gel electrophoresis was done with the PCR products.RFLP was produced and checked for the genotype.For verification of the result sequencing PCR and post clean up were also done.Normal controls were also analyzed as above.Statistical methods were adopted to compare the results. Whole blood is drawn into anticoagulants containing EDTA.There are three major steps in a PCR, which are repeated for 30 or 40 cycles in an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time. During denaturation, the double stranded DNA melts open to single stranded DNA, and all the enzymatic reactions stops.Annealing can attach and start copying the template on the double stranded DNA having the template and the primers.The primers where there are a few bases built in have a stronger attraction to the template created by hydrogen bonds than the forces breaking these attractions. Primers having no exact contact with the template do not give an extension of the fragment.The bases are coupled to the primer on 3' side,the polymerase adds dNTPs from 5' t0 3',reading the template from 3' t0 5', and the bases are added complementary to the template. Gradient PCR was done in order to standardize the PCR. Temperature gradient was applied to standardize the annealing temperature of the primers.

RESULTS

The result of the present study entitled as Role of Serotonin transporter gene in Schizophrenia are presented as follows.

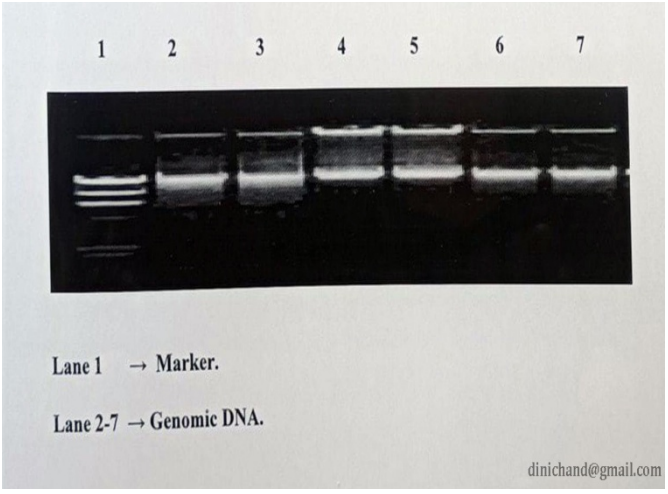

Fig 3. ***Isolated dna samples***

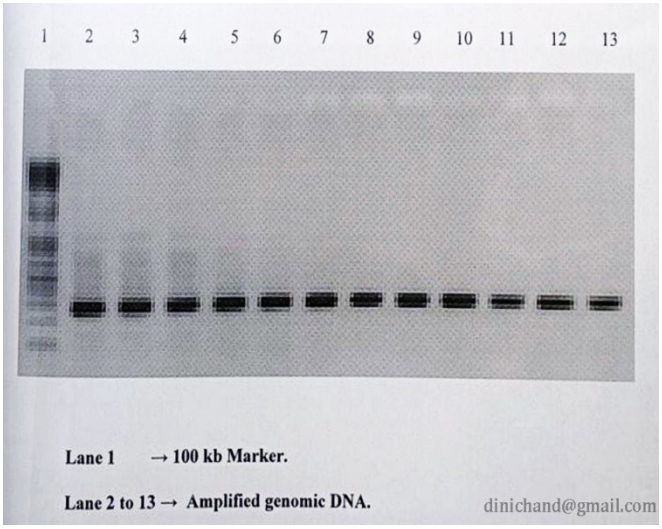

Fig. 4. ***Rapd analysis of slc6a4 gene***

400 schizophrenic patient samples and 200 control samples were selected for the Serotonin transporter gene (SLC6A4) screening. Blood samples of patients were collected from SCARF, Chennai and MHC,Trivandrum. The extracted DNA samples were subjected to RAPD analysis. The size of the polymorphic fragment to be amplified is 270kb.The isolated DNA samples and RAPD analyzed samples were illustrated in fig 3 and 4 respectively.

RAPD analyzed samples were subjected to sequencing PCR and post clean up reactions. The resulted samples were then subjected to sequencing analysis of the specified SNP polymorphisms of SLC6A4 gene .The respective sequence data analysis is illustrated in fig 5 and 6 respectively. Typical genotypic frequency were clearly visualized in the figures.

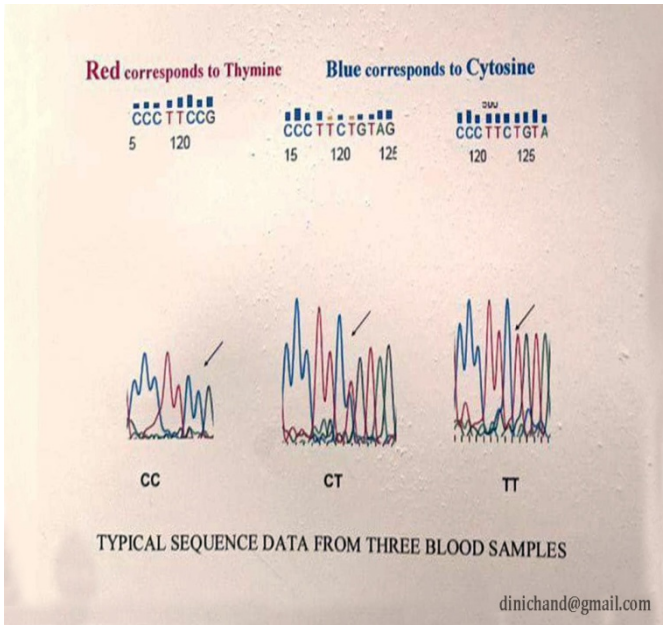

Fig. 5. ***Typical Genotypic Frequency Analysis***

Each of the patient samples and control samples were subjected to statistical analysis for the verification of statistical significance. The statistical analysis data conformations can be obtained from table 1 and table 2 respectively. In statistical analysis Hardy-Weinberg equilibrium, Genotype frequency and allele frequency and Chi-Square test were determined. The genotype frequency and allele frequency of patients and control samples were compared by Chi-square analysis. Genotype frequency distribution graph were illustrated in figure. The statistical analysis were carried out using online analysis.

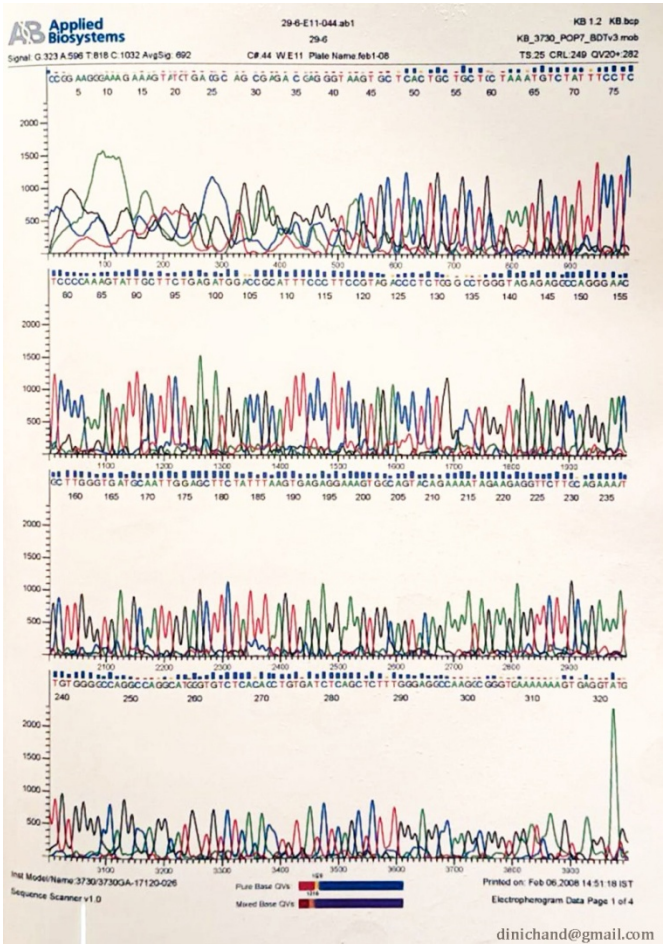

Fig. 6. ***Sequence data analysis of slc6a4 gene***

Table 1. Cases vs Control

Gene	Allele	Allelefrequency		Chi-Square	P Value
		Patients	Control		
SLC6A4	T	182	146	12.623	0.000
	C	238	314		

Table 2. Cases vs Control

Gene	Genotype	Genotype frequency		P Value
		Patients	Control	
SLC6A4	TT	42	32	0.001
	CT	98	82	
	CC	70	116	

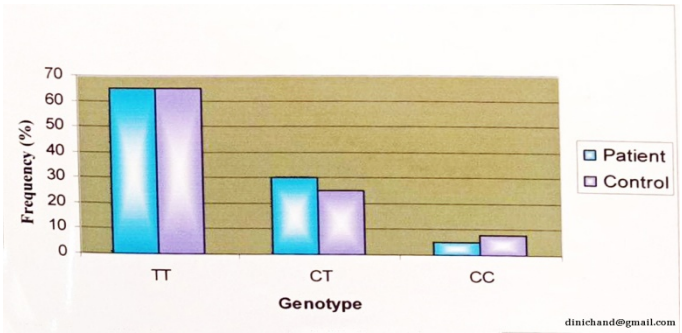

Fig. 7. ***Genotype frequency distribution***

DISCUSSION

The result of this study furnishes the evidences that the serotonin transporter gene is a primary candidate that have a major role in Schizophrenia.From the patient samples DNA were isolated using the

specific DNA isolation method. The total size of the fragment that were subjected to amplification in the present study was 270kb.By using the specific SLC6A4 gene primers, the amplification were done.These specific amplified part of the SLC6A4 gene were analyzed in the sequencing PCR reaction using specific forward primers for evident accuracy of the SLC6A4 gene polymorphisms. After sequencing PCR, the target gene were subjected to Post clean up reactions. This post clean up reactions were mainly aimed for the accurate sequencing of the specified single nucleotide polymorphisms of the target gene, SLC6A4 gene.The post cleaned samples were specifically sequenced by using the Sanger's Dideoxy method. From the sequence analysis data the SNPs of SLC6A4 gene were determined.The polymorphisms of cytosine and thymine intensity were obtained from this sequence analysis data.Patients and controls were categorized as heterozygous and homozygous based on this sequence analysis data.Whole patient samples and control samples were subjected to statistical analysis for the verification of statistical significance.In statistical analysis, Hardy –Weinberg equilibrium gives the probability of both patients and control. Both the genotype frequency and Allele frequency of patients and controls were compared by Chi-square analysis.The statistical analysis were carried out using online statistical analysis.There were significant difference in the Genotype frequency between patients and controls (P value =0.001). Comparison of Allele frequencies revealed significant difference in patients and control subjects.(P value =0.000).On line statistical analysis value were set at P = 0.5. From Genotype frequency analysis P Value were obtained as, P =0.001.Observed P value was less than the online statistically set value of P ie, P< 0.5.From this observation we can find out that the present study was statistically significant. The present study reveals the role of serotonin transporter gene due to the single nucleotide polymorphisms in Schizophrenia. The present study thus clearly indicates the involvement of SLC6A4 gene polymorphisms in schizophrenic patients from South India.As a result this finding is compatible with the previous association studies of SLC6A4 gene polymorphisms. (Lesch et al., 1998; Heils et al., 1995). A previous investigation of schizophrenic patients revealed that the role of serotonin in the pathophysiological depressions that were mainly focusing on the serotonin transporter (Owens et al., 1994). Joyce et al., 1993, demonstrated the serotonin uptakesites and serotonin receptors are altered in the limbic system of schizophrenics.Malhotra et al. 1998 demonstrated the role of serotonin transporter (5-HTT) polymorphism is associated with psychosis in neuroleptic-free schizophrenics.These findings suggest that the SLC6A4 gene is associated with the pathogenesis of Schizophrenia.

CONCLUSION

Schizophrenia is a complex mental disorder due to the involvement of several genes.Schizophrenia is mainly due to the abnormal brain function. This abnormalities is mainly involved in the transferring and processing of information's in the brain. Nerve cells in the brain communicate with each other by releasing chemicals from the nerve endings.These chemicals are called neurotransmitters. Many of the symptoms of Schizophrenia have been linked to the abnormal activity of particular neurotransmitters. Serotonin is a type of neurotransmitter that have a major role in Schizophrenia. Serotonin transporter gene (SLC6A4) is one of the most promising candidate gene in the psychiatric disorder, Schizophrenia. Serotonin transporter gene encodes an integral membrane protein that transports the neurotransmitter serotonin from synaptic spaces into presynaptic neurons.

A single nucleotide polymorphism(SNP) in the promoter of this gene has been shown to affect the rate of serotonin uptake and then cause the abnormal brain function. In the present study, the investigation is mainly based on the potential influence of the SLC6A4 gene on the psychopathology of schizophrenia due to the SNPs in SLC6A4 gene. Both the patient samples and control samples were subjected to respective DNA isolation,RAPD analysis by PCR using specified SLC6A4 gene primers.For the specified amplification of SLC6A4 gene sequencing PCR using specified forward primer alone were used and post clean up reactions were done for specified sequencing of SLC6A4 gene. From the data's obtained from sequence analysis, there were clearly observed the SNPs of SLC6A4 gene. These sequence analyzeddata's were then subjected to the statistical analysis for further clarification of the results. Data's obtained from the statistical analysis it is significantly observed the role of serotonin transporter gene in the most distressing and disabling mental disorder, Schizophrenia.

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Association of a Functional Polymorphism in the Serotonin Transporter Gene with Abnormal Emotional Processing in Ecstasy Users. *(Roiser et al. March 2005).*

Childhood experiences of abuse or trauma have also been implicated as risk factors for a diagnosis of schizophrenia in later life (Harriet et al., .2001; Schenkel et al., .2005; Janssen et al., .2004).

DNA microarrays (DNA chips) can be used to detect the presence of SNPs quickly and affordable.A single micro array can be used to screen 1, 00,000 SNP s within hours. (Gors et al., .1998).

Electroencephalograph (EEG) recordings of person with schizophrenia performing perception oriented tasks showed an absence of gamma band activity in the brain,indicating weak integration of initial neural networks in the brain(Spencer et al.,.2004).

Environmental factors:-Considerable evidence indicates that stressful life events cause or trigger schizophrenia (Day et al., .1987).

Evidences suggest that genetic vulnerability and environmental stressors can act in combination to result in diagnosis of schizophrenia (Owen et al., .2005).

Factors such as poverty,discrimination,unemployment and living in urban environment also appear to be involved in increasing the risk of schizophrenia (Van et al., .2004).

Functional SNPs that occur in the regulatory regions of the genes can affect the expression of the drug target of the gene (Cargill et al., .1999).

Genetic polymorphisms of serotonin and dopamine transporters in mental disorders. (Ueno et al.,. 2003). A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter. (Anguelova et al., .2004).

Prenatal brain development: - There is significant evidence that prenatal exposure to infections increases the risk for developing schizophrenia later in life,providing additional evidence for a link between in utero development pathology and risk of developing the condition (Brown et al., .2006).

Psychological trauma in the mother may also have an effect (Huttunen et al., .1978).

Psychosocial factors: - A number of cognitive biases or deficits have been found in people with schizophrenia (Sitskoorn et al., .2004).

Since several brain neurotransmitter receptors are involved in alcohol action, plausible candidates are neurotransmitter genes or neurotransmitter synthesis genes (Gordis et al.,. 1990).

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