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Analysis of Glycine Receptors Gene Polymorphisms in Patients with Schizophrenia

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Schizophrenia is a complex and debilitative mental disorder. There are some evidences indicating that there is a genetic linkage between Glycine receptors gene and schizophrenia incidence. This project evaluated the linkage between the three SNP of Glycine receptors gene in schizophrenia patients. A total of 113 patients including 16 women and 98 men were studied. The control group consisted of 117 healthy individuals (9 women and 108 men) with a mean age of 46.70+11.716 years, who was unrelated to SZC patients. We tried to select healthy individuals among people without any mental disorders. Results of all rs2229963¡ rs111946619 and rs78179793 SNPs showed that there is not any significant linkage between the incidence of this single nucleotide polymorphisms and risk of schizophrenia(rs2229963, P=0.934, rs111946619, P=0.780 and rs78179793, P=0.393). We conclude that those SNPs are not suitable candidates to be considered as causes for schizophrenia incidence.

Key words: Schizophrenia, Glycine receptors gene, Polymorphism.

The history of our evolving understanding of the pathophysiology of psychiatric disorders is complex and affected by the changing concepts of psychiatric disease and advances in neuroscience[1, 2]. Schizophrenia is a devastating mental disorder and roughly 1% of the world population suffers schizophrenia. The evolution of symptoms occurs in young age, the second and third decades of life. It is a hereditary disease and studies indicate that close relatives face a quadruple higher risk. Urban life is a schizophrenia risk factor and men are 30% at a higher risk than women.In men schizophrenia usually starts between 15-24 years old which means 3-5 years earlier than women. Women experience the second onset at age 45-54 which is said to be related to Estrogen level[3, 4]. Numerous studies indicated that aggression and homicide are more frequent in schizophrenia than in the general population. Individuals with a major psychiatric disorder, most commonly schizophrenia, commit 5.2% of severe acts of violence[5].Keapline and Bluer were the first to suggest schizophrenia is a mental disorder which needs better recognition. Now research focuses on three regions of the brain: frontal cortex, Thalamus and medial temporal lobe[6].Study is limited about schizophrenia due to many reasons. Defect in schizophrenia permeability and common symptoms of the disorder can be the result of drug abuse or other diseases. In addition, schizophrenia threshold limit is uncertain and can be related to place in one family but irrelevant in another[7].The first valid document about genetics relation with schizophrenia was provided by Sherrington et al. in 1988. But it can't be repetitive in independent researches. As a result, research on the links reveals some Chromosome regions 1q21-22, 1q32-41, 4q31, 5p13-14, 5q22-31, 6p22-24¡ 6q21-22, 8p21-22, 9q21-22, 10p11-15, 13q14-32,15q15, 22q11-13 æ Xp11 as the volunteer location for schizophrenia[8].

Many dopamine neurotransmitters (dopamine, glutamate, serotonin, GABA) and the interaction between many of the brain regions

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(Thalamus, Hippocampus, Frontal cortex) seems to affect schizophrenia neuropathology[9].The major brain regions related to schizophrenia are: Limbic system, Frontal lobes and basic nodes. The three regions are reciprocally connected in a way that the disorder in one region may relate to a primary damage to another region. Thalamus and brain stem are into play because of an integrative role of Thalamus and the fact that brain stem and medial section are the first station of upper motor aminergic neurons. Therefore, brain imaging of live people and Pathological investigation of brain tissue after death indicates that limbic system is the most probable station of primary pathological procedure at least in schizophrenia patients and most of the schizophrenia patients[10]. Studies have suggested that schizophrenia might result from a neurotransmission hypofunction of glutamatergic and N methyl–d-aspartate (NMDA) receptors[11-13]. In a major paradigm shift on the etiology of schizophrenia, it has been proposed that abundant genetic and environmental risk factors Together on the N-methyl-D-aspartate receptors (NMDAR)-mediated glutamatergic system and result in NMDAR hypofunction in the limbic system during neurodevelopment

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Glycine is an inhibitory neurotransmitter in the CNS and acts as a N-methyl-d-aspartate (NMDA) glutaminergic receptor potential of increasing growth hormone and intervenes in the proper function of immune system. It also plays an important role in relieving and improving the damaged tissue. Glycine (2gr a day) along with other neuroleptics is an additional therapy for schizophrenia. Glycine as an inhibitory eurotransmitter is potential of managing neurotic disorders, Hyperactivity, convulsion, depression and bipolar disorder (phase mania)[15]. Glycine can easily pass blood brain barrier and affect the center of the brain. Generally, Glycine affects its second receptors and acts as a medium of responding. The first recognized receptor of Glycine which is a strychnine sensitive receptor is located in the spine and lower that the brain stem[16, 17]. Glycine is an inhibitory neurotransmitter in the brain and spine. Glycine receptors have to subunits of Glycine-A and Glycine-B. The subunit Glycine-A includes units a and b. Again the subunit a includes a1, a2, a3 and a4 isoforms. A b unit acts as a junction Glycine receptors to sub synaptic sytoskeleton which is facilitated by sytoplasmic proteins called gephyrin[16]. On the other hand, the sub unit of Glycine-b is a receptor that is not sensitive to strychnine which acts as a facilitator in the location of Glycine attachment to glutaminergic[18]. Glycine acts as a medium by its Glycine-A activity. It also is essential for Ca prevalence NMDA receptor activity along with Glutamic acid[19].

MATERIALS AND METHODS

Study subjects

The case -control study was carried out from December 2012 to September 2013 in mental hospital in EsfehanProvince. Therelatedstudies, twoindependent samples were examined: the cases group included 114 patients with schizophrenia (98 men and 6 women)with the average age of 43.29± 12/13 years. The control group including 117 healthy individuals (108 males and 9 females) with the average age of 43.2±12.32 years.

DNA isolation and polymerase chain reaction (PCR)

For genotypic analysis, blood samples were collected in Na-EDTA tubes for DNA extraction then stored at –20 °C. carried genomic DNA extraction from blood samples. Polymorphisms were identified by PCR using the Tetra Amplification Refractory Mutation System (ARMS), a simple and rapid detection method for different types of mutations.Amplification of rs78179793, rs111946619 and rs2229963 were set according to the following PCR conditions: 10 µl of premix, 1.5µl of DNA, 1µl of both primer (Reverse outer, Forward outer) and 2µl of both primer (Forward inner¡Reverse inner) and 2.5µl of pure water. The PCR was heated at 95°C for 10 min, followed by 30 cycles at 94°C for 30 s, annealing at 47°C, 50.8°C and 65°Cfor rs78179793, rs111946619 and rs2229963 for 30 s, extension at 72°C for 30 s and final extension by incubation at 72°C for 10 min. All of annealing temperature and primers sequence are shown in table 1.

RESULTS AND DISCUSSION

Genetic studies showed that the glycine modulators site on the NMDA receptor is a considerable target for improving cognition and associated negative symptoms in schizophrenia, also manipulation of modulating sites of the NMDAR could be successful in the treatment of schizophrenia[20, 21]. NMDARs are widely thought to be crucial in synaptic plasticity and circuit formation for pre- and early postnatal stages of brain development, otherwise known as the "critical developmental window." Numerous studies have indicated that the maturation of brain circuitry is usually coincident with the NMDAR subunit switch (e.g., NR2B-to-NR2A and NR3A-to-NR3B) that occurs at the onset of the critical period of development[1, 22].

Indeed, the NMDAR-mediated glutamatergic model provides an alternate approach for conceptualizing the brain abnormalities associated with schizophrenia[13, 23].

But according to this study we found there is not any meaningful relationship between this positions and schizophrenia. And another study by Burnet in 2008 confirms our study.

Fourth dysregu- lated NMDAR subunits are usually seen in postmortem tissue from patients with schizophrenia and in animal models of NMDAR antagonism[24-27].

JaromirHons and his colleagues in 2010 with the hypothesis that the level of negative symptoms in schizophrenia is associated with a glycine, glycine on serum levels have begun to study this disease. in this study, 50 patients and 50 healthy controlled ones were enrolled.

Table 1. Primers sequence and annealing temperature of rs78179793, rs111946619 and rs2229963

SNP	Primer type	Orientation	Primer Sequence(5'→3')	TmR°C
rs78179793	Outer primers	Forward	CTGAAAAGTTAATCAACTGTATCTGTTCCA	47
		Reverse	CACAAACTTCTTCTTGATAGCATCTCC	47
	Inner primers	Forward	CTGTCTTTATTCCGTCAGGAAGAATAC	47
		Reverse	TAAAATTAAAACGACTTTCACGAGTACCA	47
rs111946619	Outer primers	Forward	GCCCTGAGTTGGAGCTTAGATTTCATTATT	50.8
		Reverse	AGGGAATGAGTTGTAAGGGTCCTTTCTCT	50.8
	Inner primers	Forward	TTTGTTTGCTAAGACTCACCTTGCCC	50.8
		Reverse	TCTTCAAGTCCATGGGACAGGATCAA	50.8
rs2229963	Outer primers	Forward	AAACAACGTGGGATAATGGAATTGGAAAT	65
		Reverse	ATCGTTAAGACTGTGGTGATGCCCAG	65
	Inner primers	Forward	AAGTTTACCTGCATTGAGGTCAAGTTTAAC	65
		Reverse	TCAAATAATATCCCATTTGGCGTTCCCGA	65

Table 2. Number and frequency of glycinreceptor¹s SNP in cases and controls

SNP	Cases N=99	Controls N=79	OR	CI	P value
rs78179793					
CT	95(94.95)	77(97.47)			Reference
CC	4(5.05)	2(2.53)	1.62	0.2249, 18.32	0.393
CC+CT	99	79	1.01	0.6518, 1.583	0.94
rs111946619					
CT	97(96.97)	76(96.2)			Reference
CC	3(3.03)	3(3.8)	0.8	0.1022, 6.025	0.78
CC+CT	99	79	1	0.6302, 1.53	0.93
rs2229963					
CT	96(95.96)	76(96.2)			Reference
CC	3(4.04)	3(3.8)	1.263	0.1643, 9.7	0.934
CC+CT	99	79	1.25	0.1631, 9.603	0.8

In this study, a significant reduction was observed in serum levels of glycine in comparison to healthy onesand this difference was observed only in men.

In this study, Glaycen serum levels which contained all glaycein genes expression was examined. But in our study, we examined only three positions on semi-autosome of X chromosomeAnd no significant correlation was found between the three positions and schizophrenia.

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