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Research Article

No Association of the Complexin-3 Gene Polymorphism with Schizophrenia

Abstract

Background: Schizophrenia (SCZ) is a multifactorial mental disease. Whereas complex interplay of genes and environment contributes to the SCZ, the disorder has still unclear biological background. Growing amount of evidence showed that synaptic dysfunctions are contributed to SCZ etiopathogenesis.

The context and purpose of the study: Complexin-3, a presynaptic regulatory protein, represents here a special interest. This study was aimed to investigate the potential association of SCZ with rs3743487 single nucleotide polymorphism of the complexin-3 protein encoding gene (*CPLX3*). A total of 350 unrelated individuals of Armenian nationality (175 SCZ patients and the same number of age-, sex-matched healthy controls) were genotyped for the selected polymorphism using polymerase chain reaction with sequence-specific primers.

Results and main findings: According to the results obtained, the frequency and carriage of the *CPLX3* rs3743487*T allele did not differ in SCZ patients as compared to controls.

Conclusions: We concluded that the *CPLX3* rs3743487*T minor allele is not associated with SCZ in Armenian population.

Brief summary: This study suggested no association of the *CPLX3* rs3743487 polymorphism with schizophrenia, however, to clarify the role of the *CPLX3* gene in SCZ further studies with much coverage of the gene and involvement of different methods are required.

Abbreviations

CPLX3: Complexin-3 protein encoding gene; PCR-SSP: Polymerase Chain Reaction with Allele-Specific Primers; SCZ: Schizophrenia

Background

Schizophrenia (SCZ) is a chronic mental disease, which affects about one percent of the population and is characterized by delusions and hallucinations [1]. It is well known that a complex interplay of genes and environment contributes to SCZ, however, the disorder has still unclear biological background and still unknown set of disease-associated genetic variants [2,3].

Synaptic hypothesis of SCZ proposes that deficits in synaptic function and connectivity are involved in the pathogenesis of this disorder [4,5]. The presynaptic abnormalities in the neurotransmitter exocytic machinery are altered in SCZ, which can yield problems with glutamate and dopamine transmission, signaling, synapse function, and development of neural system and might be responsible for cognitive dysfunction [6]. All these symptoms were observed in patients with SCZ [7]. Our own studies revealed genetic mutations of synaptic plasticity regulators in SCZ [8-10].

Complexin-3 is a member of complexin/synaphin family of presynaptic regulatory proteins. A large number of studies of complexin function in synaptic plasticity showed that this group of proteins has three functions in fusion: activation of SNARE complexes for subsequent Ca^{2+} triggering by synaptotagmin [11], clamping of SNARE complexes preventing fusion [12]; and priming of vesicles for fusion [13].

A few studies, including our own [14], show the association of SCZ with genetic variants of *CPLX1* and *CPLX2*, coding complexin-1 and complexin-2 proteins [15,16]. However, there is no data on the involvement of complexin-3 protein in the pathogenesis of SCZ. Complexin-3 as all members of complexin/synaphin family regulates the fusion of synaptic vesicles, interacting with SNARE complex, but unlike complexin-1 and complexin-2, complexin-3 together with complexin-4 is expressed at high levels mainly in retina [17]. This fact is also of interest because the impairment of visual information processing, based on the functioning of retina cells, was found in patients with SCZ [18]. The correlation between positive symptoms and electroretinogram (ERG) abnormalities and the normalization of the ERG after symptomatic improvement suggest that photoreceptor dysfunctions are state dependent in SCZ. Also, retinal dysfunctions are specific for SCZ, as compared with bipolar disorder [19].

The present study was aimed to investigate the potential association of SCZ with the complexin-3 protein encoding gene (*CPLX3*) rs3743487 single nucleotide polymorphism (SNP) tagged using International HapMap project Tag SNP picker (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/, see the details in Methods section).

Materials and Methods

Study population

In this study 175 patients with chronic SCZ patients (male/female: 73/102, mean age±SD: 46.01±10.41 years, age of the first manifestation of SCZ: 27.65±9.4 years, duration of illness: 18.36±10.51 years), and 175 controls (male/female: 86/89, mean age ± SD: 25.3±9.2 years) were enrolled. Patients were recruited from the clinics of the Psychiatric Medical Center of the Ministry of Health of the Republic of Armenia (MH RA). All patients were diagnosed as paranoid schizophrenics by two independent experienced psychiatrists, according to the presence of the relevant symptoms and the results of the Structured Clinical Interview for DSM-IV-TR (DSM-IV-TR code: 295.30) [20]. All patients with chronic SCZ were treated with typical neuroleptic haloperidol (1mg 3 times daily, *per os)*. Controls were recruited among the blood donors of the Erebouni Medical Center MH RA with no family, past or present history of any mental disorder as determined by the non-patient version of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders [21] and were not subjected to any medical treatment known to affect the brain. Exclusion criteria for all study subjects included any serious neurological, endocrine, oncological, inflammatory, autoimmune, cerebrovascular, cardiovascular, metabolic or other disorder. All study subjects were unrelated individuals of Armenian nationality. All study subjects gave their informed consents to participate in the study, which was approved by the Ethical Committee of the Institute of Molecular Biology of the National Academy of Sciences RA (IRB #00004079).

Collection of blood samples and genomic DNA extraction

10 ml of the venous blood was collected from each patient and healthy subject. EDTA was used as anticoagulant. Genomic DNA was isolated according to the standard phenol-chloroform method [22] and stored at -30°C until further use.

Selection criteria for the CPLX3 gene SNP

The rs3743487 SNP of the *CPLX3* gene was selected was selected based on the tagging results obtained using Tag SNP picker of the International Hapmap project with the selection criteria of r^2 >0.8 and minor allele frequency (MAF) >0.2 (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/).

Genotyping of CPLX3 SNP

DNA samples of patients with SCZ and controls were genotyped for the selected SNP using polymerase chain reaction with sequence-specific primers (PCR-SSP) [23]. The sequences of specific primers designed according to the GenBank sequences (GenBank ID: 594855) for allele discrimination were as follows: *CPLX3* rs3743487 SNP: forward 5′- GCC-TAT-CTT-CTG-GTT-TCT-TCC for standard C allele, forward 5′-GCC-TAT-CTT-CTG-GTT-TCT-TCT for mutant T allele, constant reverse 5′-CTC-GTG-TGT-GTC-TGT-CTG-TG. The presence/absence of allele-specific amplicons was visualized by electrophoresis in 2% agarose gel stained with ethidium bromide fluorescent dye.

Statistical analysis

Distribution of genotypes for the *CPLX3* rs3743487 SNP was checked for correspondence to the Hardy-Weinberg equilibrium. To reveal a potential association of this SNP with SCZ, its genotype, allele (gene), and phenotype frequencies (carriage rates) in patients and controls were compared. The significance of differences between allele and phenotype frequencies in study groups was determined using Pearson's Chi-square test. The odds ratio (OR), 95% confidence interval (CI), and Pearson's p-value were calculated.

Results and Discussion

A total of 350 DNA samples (obtained from 175 chronic SCZ patients and 175 controls) were genotyped. The distribution of genotypes for the *CPLX3* rs3743487 SNP in both groups were in accordance to Hardy-Weinberg equilibrium *(p>0.05)*.

The allele and phenotype frequencies of the studied genetic variant in the groups of SCZ patients and controls are shown in Table 1. According to the data obtained, the frequency and carriers of *CPLX3* rs3743487*T allele showed no significant difference between patients and controls (0.27 vs. 0.27, p=0.869 and 0.48 vs. 0.5, p=0.747, respectively).

The mutant allele of the *CPLX3* gene rs3743487 polymorphism (Gen-Bank ancestral allele C, 3′-UTR transition C/T substitution variant) is differently distributed among populations regarding to geographical area and ethnicity. It tends to be quite polymorphic in Caucasians. Thus, the highest frequency (0.44) of the rs3743487*T mutant allele is found in Gujarati Indians in Houston, Texas (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/snp_details_phase3?name=rs3743487&source=hapmap27_B36&tmpl=snp_details_phase3).

The lowest rs3743487*T frequency is found in Utah residents with Northern and Western European ancestry (0.05). Therefore, our results obtained should be replicated in other populations.

In our study population (Armenians), the *CPLX3* rs3743487*T allele frequencies in SCZ patients and healthy subjects were both 0.27 (present data), which is closer to the *CPLX3* rs3743487*T allele frequency found in Mexican ancestry in Los Angeles, California (0.21).

Our previous data indicated association of SCZ with another member of complexin family protein, a presynaptic regulating *CPLX2* gene rs1366116*T variant represents a risk factor of SCZ, whereas the *CPLX2* rs3892909*T variant is protective against SCZ

Table 1: Distribution of *CPLX3* rs3743487 genotypes and frequency of the mutant allele and its carriage in patients with SCZ and controls. The data is given as absolute numbers with proportions in parentheses.

Gene, SNP	Genotypes			Allele		Carriage
CPLX3 rs3743487	CC	CT	TT	C	T	T
SCZ	91 (0.52)	75 (0.43)	9 (0.05)	257 (0.73)	93 (0.27)	84 (0.48)
Controls	89 (0.5)	80 (0.45)	8 (0.05)	258 (0.73)	96 (0.27)	88 (0.5)
p					0.869	0.747
OR					0.97	0.93
95% CI					0.7-1.36	0.6-1.4

[14]. Unfortunately, up to date there is no published data on the role of complexin-3 in SCZ and related psychiatric diseases at both molecular and genetic levels. So, the present study is the first in this field. In future, we plan to evaluate complexin-3 blood plasma levels as well as explore potential association of *CPLX3* gene other polymorphisms with SCZ in Armenians.

Conclusion

Despite changes in synaptic plasticity are involved in cognitive impairment in patients with SCZ, our preliminary results does not nominate presynaptic regulatory protein encoding *CPLX3* gene rs3747487*T minor allele as a disease-associated genetic factor, at least in Armenian population. Further studies with more genes regulating synaptic plasticity are required to clarify the role of genes of complexin family in SCZ.

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