

Frequency of Serotonin Transporter Promote Gene Polymorphism in Opioid Addicts

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Received date: Jan 23, 2016; Accepted date: Apr 15, 2016; Published date: Apr 21, 2016

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Abstract

Background: The human serotonin transporter promoter (5-HTT) gene genotype was previously reported to be associated with temperament and personality traits at risk for substance abuse in the subjects with antisocial behaviour. Association studies of polymorphism in the promoter region of this gene in addiction have shown conflicting results. A common 44-base pair insertion/deletion polymorphism in the region of 5-HTT has been observed. This biallelic functional polymorphism designated long (L) and short (S) promoters, which affect 5-HTT gene expression since the short variant is associated with lower transcriptional activity. Since there is strong evidence of a disturbance in brain serotonergic transmission among antisocial, impulsive, anxiety, and different kinds of addiction, the purpose of present study was to evaluate the frequency of these genotypes among adolescents with opioid addiction.

Method: One hundred and forty opium dependent males, aged 20-50 years, entered the study, after signing informed written consent. They referred to the rehabilitation centre to treat their addiction. Three questionnaires, general, Minnesota Multiphasic Personality Inventory (MMPI), genetic and family pedigree) were filled out by experts. Information obtained from these questionnaires included their age, gender, consanguinity of parents, type of substance abuse, socioeconomic position, psychiatric disorder and family history of drug abuse and any other diseases. First, behavioural disorders were studied in addict cases. Then, polymorphic region of the human serotonin transporter gene was evaluated and compared between 31 addicts and 31 normal volunteer matched in age, sex and socioeconomic situation as controls. Whole blood sample (2ml) from each participant was collected on sterile tube and stored for subsequent extraction of DNA using phenol-chloroform method. Then DNA was PCR-amplified. The PCR products were resolved in 2.5% agarose gel. Data were analysed using chi-square test and $P < 0.05$ was considered significant.

Results: Short allele frequency in cases and controls were 0.532 and 0.387 respectively. Long allele frequency in cases and control were 0.468 and 0.613 respectively. Despite of OR =1.8 this study indicate no significant association between frequency of short allele and addiction to opioid.

Conclusions: Although previous findings indicated association of short allele with addiction, this study did not confirm these results. Several possibilities exist for these discrepant results, including small sample size, potential population stratification, and categorical phenotypes.

Keywords: Opioid addicts; Polymorphism; Serotonin transporter promoter

Introduction

Long term use of drugs leads to the brain deformation, followed by some abnormal behaviour [1]. The molecular bases of the addictions are complex and show no obvious pattern of Mendelian transmission [2]. Etiology and identification of involved factors in addiction are particularly important for different aspects. It is tempting to consider that addictions are polygenic [3,4], while these genes act as a complex network and may interact with each other. Furthermore, other factors such as environmental variants, factors that influence on gene expression [2-5], like methylation of promoter region of some genes, and incomplete penetrance is poorly understood.

Some specific alleles of opioidergic system gene may be involved in opioid addiction like the opioid receptor and prodynorphin genes. The 5HTT system has received considerable attention in attempts to understand the determinants of different kinds of addiction. Neurotransmitters are chemicals that communicate among nerve cells, Serotonin, also known as 5-hydroxytryptamine, is one of the important brain neurotransmitters used by many neurons [6,7].

Serotonin is a signaling molecule with a widespread effect in the CNS [5] and has a very important role in different aspects of mammalian life, like food intake, emotion, mood, respiration, pain sensitivity, cardiovascular regulation, sexual behaviour, learning and memory, circadian rhythm, sensorimotor activity, and cognition [8]. Apart from its role in development, there is evidence that disturbances in central serotonin (5-HTT) function have a specific role in

neuropsychiatric disorders, and in autism [9-11] like, impulsive aggression, violence, and criminality and many different phenomena, including alcoholism, clinical depression, obsessive-compulsive disorders, and hypertension [1,12-15]. Therefore genetic polymorphism seems to be involved in the biological vulnerability for psychoactive drugs use and dependence [16-18].

The serotonin transporter is a monoamine transporter protein. It transports and directs reuptake neurotransmitter serotonin from synaptic spaces into presynaptic neurons [7,19]. Research has shown that the repeat length polymorphism in the promoter of this gene is involved in serotonin uptake. The 5-HTT gene is located on chromosome 17q11.2, the 44-base pair insertion/deletion polymorphism in the promoter re-join of human serotonin transporter sequence localized 1.2 kb upstream the gene. The most frequently polymorphisms observed in the 5'-HTTLPR alleles are the short and long variants; the short variation has 14 Repeats of a sequence and the long variation has 16 repeats [5,20-22]. The short allele found to be associated with a lower level expression of the gene [1,10,20,22], while the activity of the long variant "L" resulted in higher serotonin transporter (5-HTT) expression. The short allele is typically considered as the "risk" allele, while the findings related to the long allele are discussed in the studies of psychopathy. Some studies found the evidence for the role of serotonin in autism and addiction. In contrast with these findings, some other studies failed to demonstrate an association between serotonin transporter gene polymorphism and the personality traits at risk for substance abuse and autism [6,10,23,24]. Based on the above-mentioned reasons, the present study designed to investigate the hypothesis that the frequency of the "low activity" S allele might be higher in the opioid addiction, when compared with non-addiction people.

Materials and Method

One hundred and forty, addict men, aged 20-50 years, entered the study and signed inform written consent. They were referred to psychiatric clinic to treat their addiction. Three questionnaires (general, MMPI, genetic and family pedigree) were completed for all

Age (years)	20-29	30-39	40<	total	Age average
With family history	7 (11.6%)	31 (52.7%)	22 (36.7%)	60 (100%)	36.5
Without family history	26 (32.5%)	32 (40%)	22 (27.5%)	80 (100%)	34.4

Table 1: Age of addicts with and without family history, Chi-square test (p-value <0.05).

Depression and anxiety were the most common psychiatric problems in the first group, while in the second group, affective disorder, passive aggressive and somatization were more common.

The average age of addicts with family history of addiction is significantly lower than addicts without family history.

Psychological disorders are significantly higher among addict individuals without family history in comparison with addict individuals with family history.

The 5-HTTLPR genotype frequencies are shown in Table 2. The frequency for LL, LS and SS for the total are 35.5%, 37.1% and 27.4%, respectively. The proportion of the SS genotype was not significantly

participants. The general questionnaire and MMPI were filled by the participants, after it was explained for them that how to do it and the genetic and family pedigree were filled by a genetic expert. Information obtained from these questionnaires included; age, gender, consanguinity of parents, type of substance abuse, socioeconomic position, psychiatric disorder and family history of drug abuse and any diseases. In the second part of investigation 2ml blood samples were taken from 31 addicts and 31 normal matched volunteers for genetic test.

Genetic evaluation

Two ml of peripheral blood from each patient was collected in sterile tubes and stored for DNA extraction using phenol-chloroform method. The 5-HTT promoter region was amplified by polymerase chain reaction (PCR). The PCR reaction was performed in a 25 ml volume containing approximately 20 ng of genomic DNA, 200 mM of each dATP, dTTP, dGTP and dCTP, 50 mM dITP (20-Deoxyinosine 50-triphosphate), 0.25 mM of each primer, 50 mM Tris/HCl (pH 8.8), 12.5 mM (NH₄)₂SO₄, 10% dimethyl sulfoxide, 6.25 mg BSA, 1 mM MgCl₂, and 2.5 U of Taq Gold DNA polymerase. After an initial denaturation step at 94°C for 10 min, the cycling parameters were 45 cycles with denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. The PCR products were resolved in 2.5% agarose gel containing 50 mg/ml ethidium bromide in TAE buffer (40 mM Tris-acetate, 1mM EDTA pH 8.0). Each gel contained one lane of 50 bp ladder to identify the 450 bp fragment designated as L and the 406 bp fragment designated as S. Data were analysed using chi-square test and P < 0.05 was considered significant.

Results

The obtained results showed that the mean age of the addict persons who had family history of addiction was lower than the addicts without family history (Table 1). Borderline personality disorder was the most common psychiatric problem (30.5%). However, for the others schizoid personality was the most common disorders (35.9%).

more consistent in addicts' samples. The allele frequencies are shown in Table 2. The frequency of the low activity allele (S) in addicts was 53.2% and in controls it was 38.7%.

The frequency of the alleles and genotypes was not different in cases and controls. However, the odd ratio was 1.8 respectively and p-value > 0.05

In spite of the fact that OD = 1.8, the results of the present study suggested that the SS genotype of 5-HTTLPR might be involved in 5-HT reuptake derangement, associated with increased psychobiological vulnerability for substance abuse.

	SS		LS		LL		Total
	Number	%	Number	%	Number	%	
Addicts	11	33.3	11	33.3	9	27.4	31
Control	6	19.4	12	38.7	13	42.9	31
Total	17	27.4	23	37.1	22	35.5	62

Table 2: 5-HTTLPR genotypes frequencies in addicts and controls.

Discussion

Genetic factors have a non-specific but significant effect on the risk of drug dependence. Some family, twin and adoption studies support this genetic role [2,23,25]. It has also been stated that different drug dependencies may have unique genetic [26]. The relationship between the 5HTT gene and dependence has been widely studied and the short allele of 5HT system has received considerable attention in addiction. Although the findings remain equivocal in other studies, it showed no

evidence for association [24,27,28], while in some studies a positive association was found [6,29]. Some recent evidences indicate that the association of addiction with short allele may be different in men and women, while it was observed to be homozygote for the short allele among males, this was heterozygote among women. This means that a genotype sex interaction was observed [4] while some other studies deny this relationship [10].

Behavioural disorder	With family history		Without family history		p-value
	Number	Percent	Number	Percent	
Major depression	6	10	8	10	0.286
Anxiety	24	40	20	25	0.364
Passive-aggressive	6	10	2	2.5	0.157
Antisocial	18	30	12	15	0.273
Borderline	27	45	36	45	0.257
Affective	3	5	10	1.25	0.033
Schizoid	18	30	34	42.5	0.027
somatization	6	10	14	17.5	0.074
accordance	0	0	4	5	-
Total	114		156		0.011
Without problem	12	20	2	2.5	0.08

Table 3: Frequency of behavioural disorder among addicts with and without family history.

In the present study we found no significant difference in rate of short allele in opioid addicted people in comparison with control cases (Table 3 and 4). More recent researchers have found evidences of psychological disorder as a factor that increase risk of dependence but according to data from present study (Table 2), consumption of drug is not associated to psychological distress and some other investigation confirmed this result. Our data indicate that the average onset age of addiction in people with family history of addiction is significantly lower than this age in addicts without family history (Table 1). Furthermore individuals with family history of addiction are more likely at risk for drug abuse in comparison with individuals without this family history. These findings highlight the importance of genetics

and inheritance in opioid addiction. The present findings need to be interpreted with caution: some unmeasured confounds may have reduced the strength and the validity of the study. Several possibilities exist for these discrepant results, including small sample size, inter individual and cultural variability, potential population stratification, and categorical phenotypes. Several studies also illustrated the importance of others moderating factors such as environmental factors. Understanding of possible associations of these variants would bring progress in principles and treatment, prevention and identification risk of addiction. Therefore further studies are required to determine whether any association is mediated by factors such as personality and sex etc.

	L-allele		S-allele		Total
	Number	%	Number	%	Number
Addicts	29	46.8	33	53.2	62
Control	38	61.3	24	38.7	62
Total	67	54	57	46	124

Table 4: Frequencies of S and L alleles in addicts and controls.

Acknowledgement

The authors acknowledge with grateful appreciation the kind assistance and financial support provided by the Vice Chancellor for Research at the Shahidsadoghi University of Medical Sciences (Yazd, Iran).

Conflict of interest

There is no conflict of interest associated with this study.

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