Polymorphism of *Prodynorphin* promoter is associated with schizophrenia in Chinese population[1]

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ABSTRACT

AIM: To investigate the correlation between single nucleotide polymorphisms (SNPs) of functional candidate gene *Prodynorphin* (*PDYN*) and schizophrenia. **METHODS:** SNPs in the promoter and exon regions of *PDYN* were screened and genotyped for association study in a cohort of Chinese Han schizophrenia cases and controls. **RESULTS:** Two SNPs *PDYN*-1576C>T and *PDYN*-946C>G were identified in the promoter region but *PDYN*-946C>G showed significant differences of allele distribution (χ^2=6.15, *P*=0.013) and genotype distribution (χ^2=6.87, *P*=0.032) between schizophrenic and control subjects. **CONCLUSION:** *PDYN*-946C>G polymorphism demonstrated an association with population susceptibility to schizophrenia (*P*<0.05).

INTRODUCTION

Schizophrenia is one of the most common mental illnesses, with a lifetime prevalence of 1 %. This disease should be caused by a combination of multiple genes and nongenetic components, or by the epistasis model in which a few genes acting jointly cause the illness. Thus, candidate genes identified on the basis of biochemical and pharmacological evidence are being tested for linkage and association studies.

Previously, most association studies of schizophrenia have focused on neurotransmitters such as serotonin and dopamine pathways but the role of neuropeptides has seldom been explored. Experimental and clinical studies suggest an involvement of the opioid neuropeptide system in schizophrenia. Opioid peptides are a family of neuropeptides which derive from three precursor gene products: proenkephalin A, proopiomelanocortin and proenkephalin B (prodynorphin, PDYN)[1]. In particular, prodynorphin, the precursor of the dynorphin opioid peptides, has been shown to play an important role in several aspects of human diseases and complex traits, eg, drug abuse, epilepsy, and mood disorders[2]. In human brain, *PDYN* is highly expressed in limbic-related areas such as the amygdala, hippocampus, ventral striatum, patch compartment of the dorsal striatum, entorhinal cortex, and hypothalamus[3], while nerve terminals and fibers containing these peptides are found in SN, globus pallidus, and raphe nuclei, *etc*. Pep-

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tides derived from prodynorphin, such as α-opioid receptors[4]. Studies indicate that *PDYN* changes its expression level under several pathophysiologically important conditions. The prodynorphin mRNA expression was increased in the patch *vs* matrix compartment of the caudate nucleus in suicide subjects and reduced in discrete nuclei of the amygdaloid complex in subjects with major depression or bipolar disorder[1,5]. Also the dysphoric effects of marijuana was found to be mediated by endogenous opioid peptide[6].

In this work, we investigated the correlation between *PDYN* and schizophrenia.

MATERIALS AND METHODS

Subjects A total of 250 unrelated schizophrenics (mean age of onset=28.2±8.0) were recruited from Shanghai Mental Health Center. All the patients had two or more of the following characteristic symptoms and each lasted a significant portion of a month (or less if successfully treated): delusions; hallucinations; disorganized speech; grossly disorganized or catatonic behaviour and negative symptoms, ie, affective flattening, alogia, or avolition. Consensus diagnosis of each patient was made by two independent psychiatrists based on DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) criteria for schizophrenia (American Psychiatric Association, 1994). Two hundred unrelated healthy subjects (mean age=31.2±8.0) from Shanghai were used as controls. These samples were recruited in medical examinations of faculty or general staff of Shanghai Mental Health Center and Shanghai Jiao Tong University. All of them were interviewed to exclude any history of psychiatric disorders. Informed consents were obtained for all the individuals tested. Thirty-two samples comprised of 16 cases and 16 controls were used for SNP screening.

Gene screen The genomic DNA sequence of *PDYN* was downloaded from Golden Path (http://genome.ucsc.edu/goldenPath/hgTracks.html) for primer design using Vecotr NTI suite6 (InforMax, Inc) and primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi/; Tab 1). All the promoter and exon regions of the gene were screened with the primers.

PCR amplification PCR was performed in a total volume of 25 μL containing 10 ng genomic DNA, 1×buffer, $MgCl_2$ 1.5mmol/L, dNTPs 0.2 mmol/L, 8 pmol of each primer, 1 unit Hotstar polymerase, in a thermal cycler (GeneAmp 9700, Appllied Biosystem). Conditions for PCR were as follows: 95 ºC for 12 min, followed by 35 cycles of 94 ºC for 30 s, 58 ºC for 30 s, 72 ºC for 30 s, and finally 72 ºC for 5 min. The PCR product was visualized on a 1.5 % agarose gel stained with ethidium bromide.

Sequencing of amplicon PCR products were purified with 96 multiscreen filter plates (Millipore Inc). Amplicon was sequenced on sequencer ABI 3100 using individual PCR primer with Big Dye version 3 (Applied Biosystems).

Sequence data processing and SNP identification The Phred and Phrap software programs were used for base calling and assembly of the chromatograms. Assemblies were edited and reviewed in Consed. Edited and complete assemblies were processed using PolyPhred software with a quality threshold of 20 to identify putative SNPs. Chromatograms were visually inspected to cull false-positive SNPs. The genotype of each SNP was visually confirmed by inspection of the chromatograms.

Statistical analysis χ^2 value, *P* value and odds ratio were calculated by SPSS 10.0 and InStat 3.05. Hardy-Weinberg equilibrium analysis was conducted using Arlequin.

RESULTS AND DISCUSSION

We identified 2 SNPs in the promoter region of *PDYN* (Tab 1) for association study in a cohort of Chinese Han schizophrenia cases and controls. Due to the positive result with *PDYN* -946C>G, we studied it in detail. Tab 2 and 3 revealed its allelic frequencies and genotypic distributions between cases and controls. The distribution of genotypes showed no deviation from Hardy-Weinberg equilibrium. In this work, we found significant differences of allele distribution (χ^2=6.15, *P*=0.013) and genotype distribution (χ^2=6.87, *P*=0.032) between schizophrenic and normal subjects.

Altered expression of *PDYN* has been observed in several experimental models of psychiatric and neurological diseases. An increase of dynorphin A peptides was detected in the cerebrospinal fluid (CSF) of drug-free schizophrenics[7], and reduced CSF levels of dynorphin (1-8) immunoreactivity was reported in patients diagnosed with schizophrenia[8]. Zimprich *et al* described a polymorphism of *PDYN* promoter: 1 to 4 repeats of a 68-bp element containing 1 binding site per repeat for the transcription factor AP1. Upon activation of the AP1 complex, H alleles (3 or 4 repeats) were associated with a significant increase in gene expression in a CAT reporter gene assay, whereas L alleles

Tab 1. PCR primers for SNP screening of the *PDYN* gene and SNPs[1)] identified.

Region	Primers	PCR product size	Polymorphisms	Flanking sequences
Promoter	5'- tggagtgatttgaggactgg	489	-1576C>T	tggccttctaT/Cgtgtgtgtgt
	5'- ctgcatttgccttttcctctc			
	5'-ccagggtggcaatgaataga	442	-946C>G	agacagggagG/Cagaaggaaag
	5'-gtagaaggggctgtttgctg			
	5'-ctgctcctggccttctatgt	518		
	5'-cacatctcaggctggcttct			
	5'-ccagggtggcaatgaataga	442		
	5'-gtagaaggggctgtttgctg			
	5'-ggatgtgatggaatgtggaa	451		
	5'-cttgaatctgcccacactga			
	5'-gagaggctgagtcccagaga	371		
	5'-tcctcatgacctagccagttg			
Exon 1	5'-ttggaggatagatggacctga	452		
	5'-tagcaggaaaaacccctcaa			
	5'-tccaggccaagggtatattg	474		
	5'-caaggagagagggacagagc			
	5'-cgtctgtctgtctgtggatca	424		
	5'-ggcctgacacaataggcttc			
	5'-caaacagcactacacccagaa	475		
	5'-tcccaccatctctaggtcca			
Exon 2	5'-tcaagaatccctccaagctc	578		
	5'-gcctgctcccttaaaatgtg			
Exon 3	5'-attcccttccttgacccttg	492		
	5'-gccatctatagggcaggaca			
Exon 4	5'-tagcagtggcgttcattttg	506		
	5'-tagcgtttgacctgctcctt			
	5'-ccatggagactggcacact	439		
	5'-aggctgggcttggatatttt			

[1)] SNPs are named according to the nomenclature recommended by Antonarakis. For promoter SNPs, the A of the the ATG of the initiator Met codon is denoted nucleotide +1. The nucleotide 5^1 to +1 is numbered -1.

Tab 2. Distribution of alleles.

SNP	Group[2)]	1	2	χ^2	*P*	Odds ratio	95 % ondidence intervasls (CI)
PDYN-946C>G	Case (*n*=223)	48 (0.11)	398 (0.89)	6.15	0.013	1.7	1.13-2.56
	Control (*n*=179)	61 (0.17)	297 (0.83)				

[2)] Due to the failure of PCR amplification, the number of samples used for statistical analysis is less than that collected.

could not be stimulated over basal conditions[2]. Ventriglia M *et al* revealed that the functional polymorphism in the promoter of *PDYN* by an epistatic interaction with the Gly allele of DRD3 gene might contribute to susceptibility to schizophrenia[9].

The opioid system has important functions in controlling pain, reward and addiction. It is also implicated in numerous other processes within and outside the nervous system. Prodynorphin transcription has been postulated as an important molecular mechanism involved

Tab 3. Distribution of genotypes.

SNP	Group	11[3)]	22[3)]	12[3)]	χ^2	*P*	Hardy-weinberg equilibrium χ^2 (*P*)
PDYN-946C>G	Case (*n*=223)	2	177	44	6.87	0.032	
	Control (*n*=179)	6	124	49			0.18 (0.67)

[3)] Alleles 1 and 2 represent the first and second nucleotides given in the name of the SNP, respectively.

in adaptation/repair processes. Expression of prodynorphin is modulated by the levels of the second messengers cAMP and Ca^{2+}: an elevation of intracellular cAMP levels causes the increase of prodynorphin mRNA levels; Ca^{2+} release from internal stores has been found to promote an increase of prodynorphin mRNA levels. Further study demonstrated Ca^{2+}-dependent prodynorphin gene transcription was insensitive to the broad-spectrum kinase inhibitors but sensitive to agents that alter internal Ca^{2+} accumulation[10]. Traumatic brain injury (TBI), which can increase intracellular calcium levels, can transiently increase prody-norphin mRNA in the hippocampus. Moreover, dynor-phin peptide immunoreactivity is enhanced for up to 24 h after TBI, which may serve a beneficial role for memory formation and storage after TBI[11]. With regard to addiction, significant increase in prodynorphin mRNA level was observed in the hippocampal dentate gyrus after acute (cocaine) and chronic (cocaine, amphe- tamine) administration of the psychostimulants[12]. Furthermore, Dynorphin A has cytotoxic effects. It may be relevant for a pathophysiological role of dynor-phins in the brain and spinal cord and for control of death of tumor cells, which express prodynorphin at high levels[13]. Endogenous dynorphins may induce hyperacusis and can contribute to the induction, main-tenance, or exacerbation of tinnitus (the perceptual correlate of altered spontaneous neural activity occurring without an external auditory stimulusin the auditory periphery)[14].

Our finding suggests that the presence of the specific promoter polymorphism increase the susceptibility to schizophrenia in Chinese population, which supports the previous study. However, it seems that only the stimulus (phorbol ester) induces gene expression but the basal transcription rate is influenced by the number of repeat elements. Additional investigation with more advanced study should be conducted to confirm the relationship between this polymorphism and mRNA expression in order to better understand the etiology of schizophrenia.

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