Failure to Support Associations of Neurotrophin-3(NT-3) Gene Polymorphism in Korean Schizophrenic Patients*

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ABSTRACT

Though initial report from Japan showed positive association of schizophrenia with dinucleotide repeat polymorphism in the NT-3 gene, subsequent studies showed mixed results. Therefore we conducted a replication study with Korean schizophrenics and matched controls who share similar ethnic background with Japanese population. The frequency of allele of dinucleotide repeat at 147 base pairs in the NT-3 gene was slightly increased, however, failed to reach statistical significance(χ^2=1.884, df=1, p<0.170) between the two groups. These findings do not support an association of NT-3 gene polymorphism with schizophrenia in Korean sample.

KEY WORDS : Neurotrophin-3(NT-3) · Dinucleotide repeat polymorphism · Association study · Schizophrenia.

Introduction

A contemporary view on schizophrenia indicated it may be a biological disorder of genetic origin as well as central nervous system dysfunction(Michael 1996). Moreover, recent findings from neuropathological studies of schizophrenia are more consistent with neurodevelopmental disorder rather than neurodegenerative one(Weinberger 1995). Lack of gliosis(Benes et al. 1986), a failure of second trimester neuronal migration(Jacob & Beckmann 1986), and anatomical asymmetry of brain(Crow 1990) are the relevant findings which support the neurodevelopmental insult in the etiology of schizophrenia.

Neurotrophines are a family of extracellular ligands that affect the differentiation, survival by inhibition of apoptosis, and maintenance of function of neuronal cells in vertebrates(Gotz & Schartl 1994). Neurotrophin-3(NT-3) is by far most highly expressed in immature regions of central nervous system in which proliferation, migration, and differentiation of neuronal precursors is ongoing. NT-3 expression dramatically decreases with maturation of these regions(Maisonpierre et al. 1990). Thus, NT-3 is thought to be essential to the brain ontogenesis, e.g. the pathogenesis of schizophrenia, and it could be an interesting candidate gene for schizophrenia.

Recently, Nanko et al.(1994) reported an association of schizophrenia with a polymorphic marker of NT-3 gene's promoter. Some replication studies from America, England, and Japan have shown inconsistent findings(Dawson et al. 1995 ; Nimgaonkar

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et al. 1995 ; Arinami et al. 1996). Considering the similar genetic background shared with Japanese, we performed an association study of NT-3 gene's polymorphism with Korean samples.

Material and Methods

Eighty eight Korean patients(mean age 30.9±6.8, range 20 - 45, years) who met the DSM-IV(American Psychiatric Association 1994) criteria for schizophrenia and 83 normal Korean controls(mean age 27.1±4.9, range 20 - 45 years) participated in this study. All subjects were given informed consent. Only unrelated male subjects were included in this study. Healthy control subjects were selected and matched by age and sex to the patients' group.

Genomic DNA was extracted from peripheral blood according to the phosphate buffered saline standard method. Polymorphic markers in the promoter region of NT-3 gene was amplified by a polymerase chain reaction(PCR)(Hattori, et al. 1993). The primer sequences were 5'-GGCTTGTGTCTTCCCCAAAGTT(CA strand) and 5'-AGGGGAGGAGGTGGAGAA. Amplification was performed with an initial denaturing of 95℃ for 5 minutes(min) and followed by 33 cycles for 1 min at 94℃, for 1 min at 65℃, for 2 min at 72℃ and final elongation step for 5 min at 72℃. Analysis of PCR product were accomplished by electrophoresis using 10% polyacrylamide, 0.25% agarose composition gel and the bands were detected by silver staining. To confirm the size of PCR product, sequencing was performed and M13 was used as the standard DNA.

Chi-square test was used to compare the NT-3 genes allelic distribution between the patient and the control groups.

Result

Table 1. shows the allelic distribution of dinucleotide repeat polymorphism at the NT-3 gene's promoter in the patient and the control groups. We found 9 alleles at the NT-3 gene's locus. The 143 base pair(bp) allele was most frequent in both groups. Although 151 bp allele and 149 bp allele were more frequent in the control group than in the patient group, they failed to reach statistical significance when Bonferroni correction was applied. Comparison of allelic distribution between the patients with younger age of onset(under 20 years) and the controls also resulted in no significant association. In addition, subgrouping the patient group either by age of onset(20 years) or by homozygous genotype again failed to get any significant associations in the allelic distribution between the two schizophrenic subgroups.

Table 1. Distribution of polymorphism at the NT-3 genes' promoter in schizophrenic patient and control group

Alleles	Schizophrenics(N=88)		Controls(N=83)	
	Homozygotes		Homozygotes	
151 bp#	0	0	0	4[a]
149 bp	0	1	1	7[b]
147 bp	2	26	0	17
145 bp	2	19	0	23
143 bp	30	90	20	74
141 bp	9	23	7	31
139 bp	0	12	0	8
137 bp	0	4	0	2
135 bp	0	1	0	0
Total	43	176	28	166

= base pair
[a] χ^2=4.00, df=1, p<.041 [b] χ^2=4.50, df=1, p<.029
Following Bonferoni correction, these two(a, b) values are not significant

Discussion

Recently Dawson et al.(1995) reported association of an allele of the NT-3 gene with Caucasian male schizophrenics in England whereas Nimgaonkar et al.(1995) reported no such association with Caucasian or African-American ethnicity in the United States. Since Korean and Japanese share similar ethnic background, it would be expected in this study to support original findings from Japan reported by Nanko et al.(1994). Furthermore, we selected only male subjects to increase the homogeneity of the study population. However, Korean sample revealed no positive association of schizophrenia not only with 147 bp allele(χ^2=1.221, d.f.=1, p<.269) which is identical with A3 allele named by Nanko et al.(1994) but also with any other alleles.

Since the life time morbid risk for schizophrenia is known to be 1%(Gottesman 1991), it could be reasonable to assume that the controls are at the risk of schizophrenia because they were too young to be free from falling ill. Therefore, further comparison of the allelic distribution between the schizophrenic patients and the controls was made on the assumption that one of the control subjects became ill and that he was homozygous for 147 bp allele. However, the results were similar to the uncorrected one.

Early onset male schizophrenics were thought to be more neurodevelopmentally abnormal than female or late onset schizophrenia(Castle & Murray 1991 ; Pilowsky et al. 1993). Therefore it could be relevant to examine the presence of linkage

disequilibrium of the polymorphic markers at the NT-3 gene's promoter site in the male schizophrenic population. In this regard, we could not confirm positive association with male schizophrenics in Korean sample as reported by Dawson et al. (1995) with Caucasian male schizophrenics. Failure to find an association of the marker locus in the neurodevelopmentally high risk group could provide more powerful basis to determine genetic implication. Findings above, taken together, suggests that linkage disequilibrium is not present at the NT-3 genes promoter site in schizophrenia.

Schizophrenia is known to be heterogeneous in etiology. Selection bias of the samples, in which phenotypes not associated with neurodevelopmental derangement was included, should be taken into account. This may, in part, explain the difference of results among studies of the NT-3 gene's association with schizophrenia. In addition, phenocopy might act as another source of error in interpreting the results of genetic study of schizophrenia(Tsuang 1994). To overcome the problems arising from genetic heterogeneity in the schizophrenic disorders, use of structured interviews and development of more rigorous research diagnostic criteria for genetic study should be considered. Case ascertainment by means of such methods could promote the validity of research in psychiatric genetics in the future.

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