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Examining Dopamine D5 Receptor Gene Polymorphism among Indian Population with Cervical Dystonia

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Abstract: ***Cervical dystonia or CD, also known as spasmodic torticollis, is a form of focal dystonia.It is caused by a multitude of environmental, neurochemical, and genetic factors but the exact etiology is still unknown. In the British and Italian population human dopamine D5 receptor has been considered as a susceptible gene for cervical dystonia. To view the hypothesis that DRD5 polymorphism is associated with the suceptible to cervical dystonia, We have examined differences in allele frequencies of DRD5 polymorphism in107 Indian patients with cervical dystonia and in 107 control subjects in India. Significant association was found for alleles 2, allele 6 and allele10 of the D5 receptor microsatellite. Allele 6 was found to be significant in patients than in control subjects. This results increased twofold risk for developing the disease. Allele 10 was found highly significant in control than in patients whereas allele 2 was also found significant in control than in patients. In conclusion, the finding of a significant association with an allele in the D5 receptor gene in patient with cervical dystonia may point out a pathogenic role of this gene.***

Keywords: cervical dystonia, allelic association, dopamine D5 receptor

1. Introduction

Cervical dystonia is one of the most common form of focal dystonia is characterized by sustained, involuntary contractions of the neck muscles that result in abnormal movements and postures of the head.[1] It is also known as the spasmodic torticollis. The etiology of cervical dystonia is unknown.[2] A gene was mapped to chromosome 18p (DYT7) in a large German family in whom the phenotype was predominantly adult onset torticollis.[3] It has been observed that abnormalities of dopaminergic neurotransmission in the basal ganglia may be responsible for producing dystonic movements. Dopa responsive dystonia caused by mutations in the for GTP-cyclohydrolase I, the rare limiting enzyme for the synthesis of tetrahydrobiopterin, a key cofactor in Dopamine synthesis.[4] The DYT1 gene which causes childhood onset autosomal dominant primary generalized dystonia has recently been cloned and encodes a protein called torsinA.[5] The function of torsinA is unknown but it is preferentially expressed in the substantia nigra par compacta[6] and may interfere with dopamine vesicle transport[7]. A recent study on British and Italian patients affected by cervical dystonia showed an association between the dopamine D5 receptor and dystonia, suggesting a multifactorial inheritance of cervical dystonia.[8,9]The aim of this study, therefore, was to examine if polymorphisms in the gene for the dopamine D5 receptor gene is associated with cervical dystonia.

2. Materials and Methods

Patients

One hundred seven patients with cervical dystonia were identified from Medicine and Neurology OPD of S.S.Hopital, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Each patients had been seen by a neurologist with expertise in the field of dystonia.

Controls

One hundred seven control subjects with a similar age, range, sex and ethnic background were collected from patients attending the blood bank of S.S hospital, BHU who did not have a neurological illness. This study was ethically approved.

Collection of Material for PCR:

Blood (1-2 ml) was collected and processed either the same day for the isolation of DNA or kept at -20^0C for future use. Samples from the hospital site were brought on ice and processed in the similar fashion.

DNA Isolation

DNA was isolated by the phenol–chloroform method following standard protocol. Briefly, fresh and frozen (after thawing) blood samples were treated with RBC lysis buffer ([pH 7.4]; 155mM NH_4Cl, 10mM Potassium Bicarbonate, 0.1mM EDTA). After centrifugation the white blood cells (WBC) pellets were suspended in extraction buffer (10mM Tris HCl [pH 8.0], 0.1mM EDTA, 20µg/ml pancreatic Ribonuclease A, and 0.5% SDS). Proteinase K (100µ g/ml)

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was used in both cases. DNA was extracted by phenol – chloroform extraction and ethanol precipitation. DNA threads were washed with 70% ethanol and air dried. DNA was dissolved in 50µl TE buffer (10mM Tris HCl [pH 7.5], 1mM EDTA autoclaved).

Primers:
The following Flourecently labeled primers (Forward Primer CGTGTATGATCCCTGCAG; Reverse Primer GCTCATGAGAAGAATGGAGTG were constructed to amplifying the region containing the (CT/GT/GA)n polymorphism.[10] The forward primer was flourcently labeled with FAM.

PCR Amplification:
5µl of DNA was added to 45µl of reaction mixture containing 10mM Tris HCl (pH 8.3) and 50mM KCl, 1.5mM $MgCl_2$, 200µM of each dNTPs (Genetec, India), 50ng of each primers and 1.25U Taq DNA polymerase (Genetec, India). Amplification was performed in a thermal cycler (MJ Research, USA), with the heated lid option, programmed for 40 cycles of denaturation at 94^0C for 1 min, annealing at 45^0C for 1 min, extension at 72^0C for 2 minutes, preceded by initial denaturation at 94^0C for 2 minutes. Final extension was for 3 min at 72^0C.

Genotyping:
The amplified PCR product was run on an ABI PRISM 3100 automated sequencer (Applied Bioystem) and analyse with dedicated software.

Statistical analysis:
Statistical analysis was carried out using the χ^2 test and odds ratios (OR) with relative 95% confidence intervals (CI).

Results

Female to male ratio was 1:1.6 in patients and 1:1.3 in controls. The mean age of cases and control was 53.4 years and 45.4 years respectively.

Table1 represents the results of DRD5 (CT/GT/GA)n microsatellite genotyping in patients and controls. A total of 12 alleles were detected for the (CT/GT/GA)n microsatellite. A significant association was found for allele 6 (bp 146), more frequent in patients than in control subjects. This resulted in approximately twofold increased risk of developing the disease (OR 0.521; 95% CI 0.317 to 0.857; p = 0.01, table 1). Conversely, allele 5 (bp 148) was more common in controls than in patients, but the difference did not reach statistical significance (OR 0.922; 95% CI 0.621 to 1.369; p = 0.687). The frequencies of allele 10 (bp 138) (OR 6.815; 95% CI 2.591 to 17.929, p= 0.00) and allele 2 (OR 4.178; 95% CI 1.162 to 15.024), were significantly higher in controls than the patients.

Discussion

The aim of this study was to evaluate association between DRD5 and sporadic adult onset cervical dystonia in Indian population. In our study, the overrepresentation of allele 6 (p=0.010) of the D5 receptor dincleotide repeat within the CD patients group compared with the normal controls probably resulted in a two fold increased risk of developing the disease (table 1). Conversely, allele 2 and allele 10 were overrepresented in control subjects compared to CD patients suggested their protective role in the development of disease. However, the frequency of distribution of allele 10 (p=0.00) was significantly higher than allele 2 (p=0.018) in controls suggested more protective role for this allele as compared to other alleles. Both susceptibility and protective DRD5 alleles in the present study are different to those described in the British and Italian study [8, 9].

The DRD5 gene codes for a dopamine receptor included in the D1 super family. The involvement of a dopamine pathway in the pathogenesis of PTD is particularly entrancing. It has been recently suggested that dopamine D1/5 receptors could play a role in the so called indirect pathway of the basal ganglia circuitry.[11] Furthermore, DRD5 has been recently associated with blepherospasm, another form of adult onset focal PTD.[12] Additional facts supports a link between dopamine and dystonia. Autosomal dominant dopa responsive dystonia is caused by mutations in the GTP cyclohydrolase I gene, whose protein involved in dopamine synthesis. Parkinson's disease affected patients, when treated with L-dopa , can develop dystonic dyskinesias, and antipsychotic drugs which inhibit dopamine receptors can produce dystonia. The multifactorial inheritance of cervical dystonia has been already recommended, based on the observation of familial clustering.[13,14] The association between DRD5 and CD in three independent studies hints towards a accepted role of DRD5 as a susceptible gene for primary cervical dystonia.

Table 1: Distribution of the (CT/GT/GA) n Microsatellite alleles in 107 cervical dystonia patients and 107 controls

Allele	Patients	Controls	Odds ratio	95% CI (lower)	95% CI (upper)	P- value
1(156)	0	2	0.991	0.978	1.004	0.156
2(154)	3	12	4.178	1.162	15.024	0.018
3(152)	21	20	0.947	0.498	1.804	0.870
4(150)	16	14	0.866	0.412	1.822	0.705
5(148)	79	75	0.922	0.621	1.369	0.687
6(146)	51	30	0.521	0.317	0.857	0.010
7(144)	9	8	0.885	0.335	2.338	0.805
8(142)	7	6	0.853	0.282	2.581	0.778
9(140)	9	8	0.885	0.335	2.338	0.805
10(138)	5	30	6.815	2.591	17.929	0.000
11(136)	8	7	0.871	0.310	2.445	0.793
12(134)	3	2	0.664	0.110	4.011	0.653

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Abbreviations: CD, cervical dystonia; CI, Confidence interval; DRD5, dopamine D5 receptor, OR, odds ratio; PTD, primary torsion dystonia.

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