SHORT REPORT

Role of the dopamine D5 receptor (DRD5) as a susceptibility gene for cervical dystonia

F Brancati, E M Valente, M Castori, N Vanacore, M Sessa, G Galardi, A Berardelli, A R Bentivoglio, G Defazio, P Girlanda, G Abbruzzese, A Albanese, B Dallapiccola, and the Italian Movement Disorder Study Group

J Neurol Neurosurg Psychiatry 2003;**74**:665–666

Cervical dystonia (CD) is one of the most common forms of primary dystonia. The pathogenesis of the disease is still unknown, although evidence suggests a role for genetic factors. Recently, a polymorphism in the dopamine D5 receptor (DRD5) gene has been associated with the disease in a British population, suggesting that DRD5 is a susceptibility gene for CD. To confirm these data, we performed a case-control study of the microsatellite $(CT/GT/GA)_n$ at the DRD5 locus in 104 Italian CD patients and 104 healthy controls. The frequency of allele 4 was higher in the CD patients compared to the controls. This resulted in a twofold increased risk of developing the disease. These results provide further evidence of an association between DRD5 and cervical dystonia, supporting the involvement of the dopamine pathway in the pathogenesis of CD.

Cervical dystonia (CD), one of the most common forms of primary torsion dystonia (PTD), is characterised by sustained muscle contractions of the cervical musculature, leading to twisting and repetitive movements and abnormal postures of the neck.[1] Genetic studies have contributed to an understanding of the basis of the disease. Although the great majority of cases are considered sporadic, it has been shown that CD is more common in first degree relatives of affected individuals than in the general population, and concordance in monozygotic twins has been reported.[2 3] A genome-wide search in a large German kindred with adult onset CD led to the identification of a locus on chromosome 18p (DYT7).[4] However, linkage to DYT7 was not confirmed in other PTD families and the gene has not yet been cloned. A recent study on a cohort of English patients affected by CD showed an association between the dopamine D5 receptor (DRD5) and dystonia, suggesting a multifactorial inheritance of CD.[5] For any association study to be validated, recently published guidelines require that the results are replicated in an independent group of subjects.[6] In order to establish whether DRD5 is associated with CD, we genotyped 104 Italian patients for the $(CT/GT/GA)_n$ polymorphism at the DRD5 locus, and compared allele frequencies with control subjects.

PATIENTS AND METHODS

Patients

A total of 104 outpatients with CD were recruited from seven Italian movement disorders clinics over a period of two years. All patients had received a diagnosis of primary cervical dystonia according to published criteria[1] and reported no family history of dystonia on interview. Diagnosis was made by a neurologist trained in movement disorders and confirmed by a senior neurologist blinded to the diagnosis. Patients with secondary dystonia and familial cases were excluded. All participating individuals were requested to sign an informed consent form. Control subjects were recruited among outpatients attending the same hospitals for non-neurological conditions. Each CD patient was matched with one control for sex, age (±1 year), and geographical area of origin. The study was undertaken following approval of the local ethics committees.

Genotypic analysis

DNA was extracted from peripheral blood following standard procedures. Fluorescently labelled primers were constructed to amplify the region containing the $(CT/GT/GA)_n$ polymorphism. PCR products were run on an ABI PRISM 3100 automated sequencer (Applied Biosystems, Foster City, CA), and analysed with dedicated software. Alleles were numbered as previously described by Placzek and colleagues.[5] Two British DNA samples (courtesy of Dr TT Warner) were genotyped and allele sizes compared, to make sure the same allele numbers were assigned as in the British study.

Statistical analysis

Statistical analysis was carried out using the χ^2 test, and odds ratios (OR) with relative 95% confidence intervals (CI). Statistical procedures were performed using the BMDP New System software.

RESULTS

Female to male ratio was 1.08 in both patients and controls. The mean age of cases and controls was 49.6 (14.9) years and 49.5 (15.0) years respectively. The mean age at onset of dystonia was 45.2 (10.7).

Table 1 presents the results of $(CT/GT/GA)_n$ 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 4 (bp 150), more frequent in patients than in control subjects. This resulted in a twofold increased risk of developing the disease (OR 2.44; 95% CI 1.14 to 5.27; $p = 0.01$). Conversely, allele 10 (bp 138) was more common in controls than in patients, but the difference did not reach statistical significance (OR 0.56; 95% CI 0.30 to 1.06; $p = 0.06$).

DISCUSSION

The aim of this study was to evaluate the previously reported association between DRD5 and sporadic adult onset cervical dystonia in an Italian population. In our study, allele 4 was significantly associated with CD, resulting in a twofold increased risk of developing the disease. The frequency distribution of allele 10 in cases and controls suggests a protective

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

Table 1 Distribution of the $(CT/GT/GA)_n$ microsatellite alleles in 104 sporadic CD patients and 104 matched controls

Allele	Cases	Controls	OR (95% CI)	p value
1 (156)	3	1	3.03 (0.24 to 159.8)	–
2 (154)	3	3	1.00 (0.13 to 7.55)	–
3 (152)	20	24	0.82 (0.42 to 1.59)	–
4 (150)	27	12	2.44 (1.14 to 5.27)	0.01
5 (148)	83	76	1.15 (0.76 to 1.75)	–
6 (146)	18	9	2.09 (0.87 to 5.18)	–
7 (144)	6	8	0.74 (0.21 to 2.49)	–
8 (142)	6	14	0.41 (0.13 to 1.17)	–
9 (140)	8	15	0.51 (0.19 to 1.33)	–
10 (138)	20	33	0.56 (0.30 to 1.06)	0.06
11 (136)	10	5	2.05 (0.62 to 7.77)	–
12 (134)	4	6	0.66 (0.14 to 2.83)	–

role for this allele, but lack of statistical significance does not allow confirmation of this data. Both susceptibility and protective DRD5 alleles in the present study are different to those described in the British study. This could be explained by the fact that the $(CT/GT/GA)_n$ microsatellite is located at 5′ outside the DRD5 coding region, making a functional role for this polymorphism very unlikely. Therefore, we suggest that a functional, still unidentified variant in DRD5, identical in the two populations, could be in linkage disequilibrium with two different $(CT/GT/GA)_n$ alleles, depending on the different genetic background of the Italian and British populations.

The DRD5 gene codes for a dopamine receptor included in the D1 super family. The involvement of the dopamine pathway in the pathogenesis of PTD is particularly intriguing. 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.[7] Moreover, DRD5 has been recently associated with blepharospasm, another common form of adult onset focal PTD.[8] Other evidence supports a link between dopamine and dystonia. The protein encoded by the DYT1 gene, torsin A, mutated in early onset generalised PTD, is a member of the AAA+ super family of chaperone proteins and is highly expressed in dopaminergic neurones in human brain. Autosomal dominant dopa responsive dystonia is caused by mutations in the GTP cyclohydrolase I gene, whose protein is involved in dopamine synthesis. Patients affected by Parkinson's disease, when treated with L-dopa, can develop dystonic dyskinesias, and antipsychotic drugs which inhibit dopamine receptors can produce dystonia.

The multifactorial inheritance of CD has been already suggested, based on the observation of familial clustering.[9 10] The association between DRD5 and CD in two independent studies hints towards a putative role of DRD5 as a susceptibility gene for primary cervical dystonia. Nevertheless, these results must be interpreted with some caution and further studies are needed to confirm the involvement of DRD5 in CD and other forms of PTD. In particular it would be useful to identify the functional variant, in order to better understand the role of this receptor in the pathogenetic mechanism leading to the disease.

ACKNOWLEDGEMENTS

This work was supported by grant "Clinical and molecular genetics of movement disorders" 2001, from the Italian Ministry of Health.
Participants to the Italian Movement Disorder Study Group: S Amadio (Milano), S Barbieri (Milano), M Basciani (San Giovanni Rotondo), L Bertolasi (Verona), U Bonuccelli (Pisa), L Capus (Trieste), M C Carboncini (Pisa), P Cerrato (Torino), A Currà (Roma), M T Desiato (Roma), R Eleopra (Ferrara), R Liguori (Bologna), G Majorana (Messina), F Mancini (Pavia), L Manfredi (Como), R Marchese (Genova), D Martino (Bari), S Maurri (Firenze), F Rasi (Forlì), A Rossi (Perugia), L Santoro (Napoli), R Shoenhuber (Bolzano), P Soliveri (Milano), L Vacca (Roma), F Valzania (Bologna).

Authors' affiliations
F Brancati, M Castori, Department of Experimental Medicine and Pathology, University "La Sapienza", Rome
B Dallapiccola, E M Valente, CSS Hospital, IRCCS, San Giovanni Rotondo, CSS-Mendel Institute, Rome, Italy
N Vanacore, Laboratory of Epidemiology and Biostatistics, National Institute of Health, Rome
M Sessa, Istituto Scientifico H.S. Raffaele, Milan
G Galardi, Neurorehabilitation Unit, IRCCS Fondazione Salvatore Maugeri, Gussago
A Berardelli, Department of Neurological Sciences, University "La Sapienza", Rome
A R Bentivoglio, A Albanese, Institute of Neurology, Catholic University, Rome
G Defazio, Department of Neurological Sciences, University of Bari
P Girlanda, Department of Neurosciences, Psychiatry and Anaesthesiology, University of Messina
G Abbruzzese, Department of Neurological Sciences and Vision, University of Genoa

Correspondence to: Dr F Brancati, CSS Mendel Institute, Viale Regina Margherita, 261, 00198 Rome, Italy; f.brancati@css-mendel.it

Received 11 September 2002
Accepted 21 December 2002

REFERENCES

1 **Fahn S**, Bressman SB, Marsden CD. Classification of dystonia. *Adv Neurol* 1998;**78**:1–10.
2 **Defazio G**, Berardelli A, Abbruzzese G, *et al*. Possible risk factors for primary adult onset dystonia: a case-control investigation by the Italian Movement Disorders Study Group. *J Neurol Neurosurg Psychiatry* 1998;**64**:25–32.
3 **Wunderlich S**, Reiners K, Gasser T, *et al*. Cervical dystonia in monozygotic twins: case report and review of the literature. *Mov Disord* 2001;**16**:714–18.
4 **Leube B**, Rudnicki D, Ratzlaff T, *et al*. Idiopathic torsion dystonia: assignment of a gene to chromosome 18p in a German family with adult onset, autosomal dominant inheritance and purely focal distribution. *Hum Mol Genet* 1996;**5**:1673–7.
5 **Placzek MR**, Misbahuddin A, Chaudhuri KR, *et al*. Cervical dystonia is associated with a polymorphism in the dopamine (D5) receptor gene. *J Neurol Neurosurg Psychiatry* 2001;**71**:262–4.
6 **Bird TD**, Jarvik GP, Wood NW. Genetic association studies: genes in search of diseases. *Neurology* 2001;**57**:1153–4.
7 **Svenningsson P**, Le Moine C. Dopamine D1/5 receptor stimulation induces c-fos expression in the subthalamic nucleus: possible involvement of local D5 receptors. *Eur J Neurosci* 2002;**15**:133–42.
8 **Misbahuddin A**, Placzek MR, Chaudhuri KR, *et al*. A polymorphism in the dopamine receptor DRD5 is associated with blepharospasm. *Neurology* 2002;**58**:124–6.
9 **Defazio G**, Livrea P, Guanti G, *et al*. Genetic contribution to idiopathic adult-onset blepharospasm and cranial-cervical dystonia. *Eur Neurol* 1993;**33**:345–50.
10 **Waddy HM**, Fletcher NA, Harding AE, *et al*. A genetic study of idiopathic focal dystonias. *Ann Neurol* 1991;**29**:320–4.