Brain (1998), **121,** 2335–2339

The role of DYT1 in primary torsion dystonia in Europe

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Summary

Primary torsion dystonia (PTD) is a clinically and genetically heterogeneous movement disorder. DYT1 on chromosome 9q34 was the first PTD gene to be mapped. A 3-bp (GAG) deletion in this gene was reported to account for almost all early limb-onset generalized PTD. No relationship has been found with DYT1 in patients with prominent craniocervical involvement. To elucidate the DYT1-associated phenotype, we analysed the DYT1 mutation in 150 PTD patients, either sporadic or index cases from small PTD families. Twenty-two patients were positive for the GAG deletion in the DYT1 gene. Fifteen of them presented with the typical DYT1 phenotype (early, limb-onset generalized dystonia without spread to craniocervical muscles), four had limb-onset dystonia with spread to craniocervical muscles, two patients had arm-onset segmental dystonia and one had focal right-arm dystonia. One-hundred and twenty-eight patients were negative for the DYT1 mutation. Forty-six of them had segmental dystonia and 59 had focal dystonia. The other 23 patients presented with generalized dystonia, either with craniocervical involvement (13 patients) or without spread to the craniocervical region (typical DYT1 phenotype—10 patients). These data confirm the importance of the GAG deletion in European cases of PTD, and indicate phenotypic and genotypic heterogeneity.

Keywords: DYT1 gene; primary torsion dystonia

Abbreviations: PCR = polymerase chain reaction; PTD = primary torsion dystonia

Introduction

The dystonias are a clinically and genetically heterogeneous group of movement disorders characterized by sustained involuntary muscle contractions causing twisting movements and abnormal postures (Fahn *et al.*, 1987). The classification of dystonias has recently been revised with subdivision into four categories (Fahn *et al.*, 1997): (i) primary torsion dystonia (PTD), where the phenotype is of dystonia alone, which may be accompanied by tremor; (ii) dystonia-plus syndromes, where the dystonia may be accompanied by other neurological features such as parkinsonism (dopa-responsive dystonia) or myoclonus (myoclonic dystonia); (iii) secondary dystonias, resulting from environmental factors (such as birth trauma, anoxia and stroke); (iv) heredodegenerative diseases, where dystonia is part of a more complex clinical neurodegenerative phenotype, such as Huntington's disease and Wilson's disease.

PTD is the most prevalent form of dystonia and has a wide clinical spectrum, including generalized dystonia, segmental dystonia and focal dystonia (e.g. cervical dystonia, blepharospasm and writer's cramp). PTD has a very variable age at onset and this largely determines its severity (Fahn *et al.*, 1987). Those with onset in childhood tend to develop generalized dystonia which starts in a limb, and the majority of these are familial. In contrast, dystonia with onset in adult life usually affects craniocervical muscles and remains focal or segmental in distribution. There is increasing evidence for the role of genetic factors in the aetiology of PTD. Seven dystonia loci have been mapped, and two genes cloned (Warner and Jarman, 1998).

Early-onset generalized dystonia is the most severe form, and the majority of cases are caused by an autosomal dominant gene (DYT1) with reduced penetrance (Bressman *et al.*, 1989; Fletcher *et al.*, 1990). The DYT1 gene was linked to chromosome 9q34 (Ozelius *et al.*, 1989) both in Ashkenazi Jews, in whom the condition appears more common, and in non-Jewish kindreds (Kramer *et al.*, 1990, 1994; Warner *et al.*, 1993). In the Ashkenazi families, significant allelic association between a haplotype of

chromosome 9q34 markers and dystonia was found, suggesting a founder effect (Ozelius *et al*., 1992; Risch *et al*., 1995).

The DYT1 gene has recently been cloned and sequenced. A 3-bp (base pair) deletion in the coding sequence of the gene was found in all affected individuals and obligate carriers with 9q34-linked PTD, regardless of ethnic background and surrounding haplotype (Ozelius *et al*., 1997). The deletion results in loss of a glutamic-acid residue in a conserved region of a novel ATP-binding protein, termed torsinA.

The typical DYT1 phenotype appears similar in all ethnic populations; symptoms begin in an arm or leg and progress to involve other limbs and the trunk, sparing craniofacial muscles. Ozelius *et al*. (1992) also identified the DYT1 mutation in two patients with early, limb onset dystonia spreading to craniocervical muscles, while none of 76 families with focal or segmental craniocervical dystonia carried the mutation. The GAG deletion in the DYT1 gene does not account for all cases of typical early-onset dystonia, as five out of 19 probands screened by Ozelius did not carry this deletion.

In order to evaluate the role of the DYT1 3-bp deletion in European PTD patients, and to clarify the relationship between genotype and phenotype in dystonia, we studied 150 patients with PTD for the presence of the GAG deletion in the DYT1 gene.

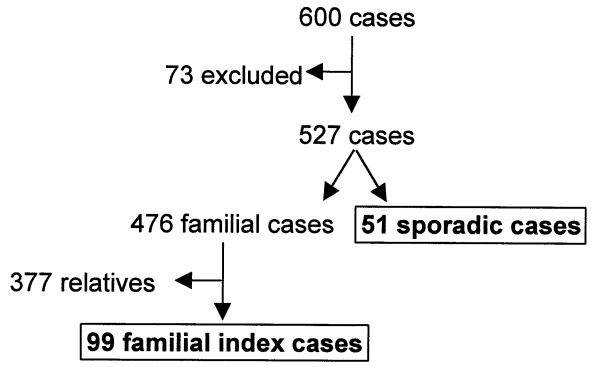

Fig. 1 Ascertainment of the 150 dystonia cases in the study.

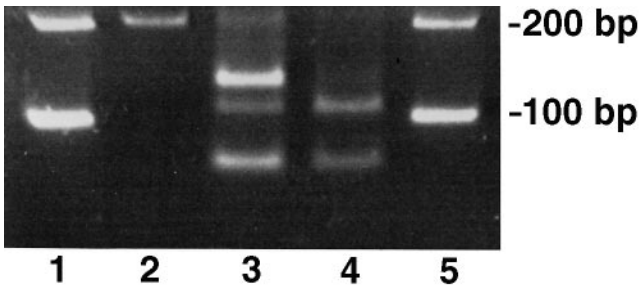

Fig. 2 Minigel picture. The digestion of PCR product with *Bse*RI generates two bands of 120 and 70 bp in a control subject (lane 4), while a novel 130-bp band is generated in an affected individual with the GAG deletion (lane 3). Lane 2 shows the original PCR fragment of 200 bp (before digestion). Lanes 1 and 5 are markers (100-bp ladder—Life Technologies).

Material and methods

Patients

Patients were selected from the dystonia database of the Movement Disorder Clinic and the Neurogenetic Clinic at the National Hospital for Neurology and Neurosurgery, London and from previous clinical and genetic studies of familial PTD (Fletcher *et al*., 1990; Warner *et al*., 1993). Inclusion criteria were: (i) generalized, multifocal, segmental or focal dystonia as defined by published criteria (Fahn *et al*., 1987); and (ii) a clinical course compatible with PTD with no features to suggest secondary dystonia or other dystonic states such as dopa-responsive dystonia or paroxysmal dystonias.

A total of 600 cases were initially ascertained, and the available hospital notes were examined. Seventy-three cases were excluded because of patient refusal, unavailability of clinical notes, uncertain diagnosis or probable secondary dystonia. In cases of familial PTD (476 patients in 99 families), only index cases were included. A total of 150 patients were included in the study (99 familial index cases and 51 sporadic cases) (Fig. 1). All patients had been examined by a neurologist from the Movement Disorder Clinic, and had blood sampled with informed consent; the majority of patients are currently under follow up at either the Movement Disorder Clinic, the Botulinum Toxin Clinic or the Neurogenetic Clinic at the National Hospital for Neurology and Neurosurgery. All patients were British except one Asian, two Italian and three French subjects; eight were of Ashkenazi Jewish ancestry, one was Sephardic Jewish and two patients had one parent of Ashkenazi Jewish origin.

DNA analysis

DNA was extracted from peripheral blood leucocytes using standard techniques. Polymerase chain reaction (PCR) was performed in a total volume of 20 ml using 100 ng of genomic DNA, 7 pmol of each primer, 200 mM dGTP, dCTP, dTTP and dATP, 1.5 mM $MgCl_2$, 10% dimethylsulfoxide and 0.5 U of Perkin Elmer AmpliTaq Gold polymerase (Branchburg, New Jersey). The primers used are those described by Ozelius *et al*. (1997). PCR conditions were as follows: one cycle at 94°C for 11 min, followed by 35 cycles at 94°C for 30 s, 49°C for 30 s and 72°C for 30 s. Then, 10 ml of each PCR product were digested with 4 U of the restriction endonuclease *Bse*RI, loaded on a 4% intermediate melting temperature agarose gel and electrophoresed at 50 V for 75 min.

The 200-bp normal PCR product contains two sites recognized by the restriction endonuclease *Bse*RI; after digestion, two fragments of 120 and 70 bp are generated. Affected individuals have a novel 130-bp fragment resulting from abolition of a *Bse*RI site by the GAG deletion (Ozelius *et al*., 1997) (Fig. 2).

Table 1 *Phenotypes of DYT1 mutation-positive and -negative patients*

Phenotype	DYT1+ [*n* (%)]	DYT1– [*n* (%)]	Total (*n*)
Generalized dystonia			
Limb onset without spreading to cranio-cervical muscles (typical DYT1 phenotype)	15 (60)	10 (40)	25
Limb onset spreading to craniocervical muscles	4 (29)	10 (71)	14
Neck onset spreading to limbs	0 (0)	3 (100)	3
Segmental dystonia			
Craniocervical onset	0 (0)	37 (100)	37
Arm onset	2 (18)	9 (82)	11
Focal dystonia	1 (2)	59 (98)	60

Table 2 *Presence of family history in DYT1 mutation-positive and -negative patients*

Phenotype	Familial [*n* (%)]	Sporadic [*n* (%)]	Total (*n*)
DYT1+ ($n = 22$)			
Generalized dystonia	17 (89)	2 (11)	19
Segmental dystonia	1 (50)	1 (50)	2
Focal dystonia	1 (100)	0 (0)	1
DYT1– ($n = 128$)			
Generalized dystonia	21 (91)	2 (9)	23
Segmental dystonia	35 (76)	11 (24)	46
Focal dystonia	24 (41)	35 (59)	59

Results

Phenotypes of mutation-positive and mutation-negative patients are summarized in Table 1. The correlates between the clinical subtypes, the presence or absence of a family history and the results of mutation analysis are shown in Table 2.

Mutation-positive cases

Twenty-two patients were found to have the GAG deletion in the DYT1 gene. All but three of these (88%) had at least one relative affected by PTD. Four were Ashkenazi Jewish, and one had a single parent of Jewish origin. The onset of dystonia was before the age of 28 years in all mutation-positive patients (mean 9.9 years, SD 4.3 years, minimum 2 years, maximum 21 years).

Fifteen patients presented with the typical DYT1 phenotype, i.e. early leg-onset dystonia spreading to other limbs but not to craniocervical muscles.

Four patients presented a mildly atypical phenotype. In three of them dystonia started in a leg and spread to the upper limbs eventually involving craniocervical muscles; in the fourth patient dystonia started in the right arm and spread to the other arm, to the legs and the cervical muscles. Two affected relatives of this patient (a maternal uncle and a cousin) had a milder phenotype with focal dystonia of the hand and neck respectively; both of them carried the GAG deletion.

Three mutation-positive patients had a clearly atypical phenotype and merit more detailed description.

Patient 1

This patient had one parent of Ashkenazi Jewish origin. He presented at the age of 10 years with segmental dystonia in the right arm, which spread up to craniocervical muscles but not to the other limbs. He had no affected relatives.

Patient 2

This individual is the index case of a non-Jewish kindred (Family W) whose pedigree is shown in Fig. 3. She is a 28-year-old female who presented at the age of 21 years with writer's cramp affecting her right arm. At the age of 23 years she developed dystonic posturing of that arm, but no further spread of dystonia has occurred. Her mother (III-2) also has atypical features for DYT1, with onset of cervical dystonia at the age of 50 years. At the age of 62 years this spread to involve spasms in her back (segmental axial dystonia). The brother of the index case (IV-1) developed dystonia of the left foot at the age of 9 years, which spread to involve both legs and arms, the trunk and larynx. However, a maternal cousin (III-1) has typical limb onset generalized dystonia, which started in the right leg at the age of 11 years and spread to involve both lower limbs, the trunk and the right arm. All affected individuals in this family carry the GAG deletion.

Patient 3

This 35-year-old man, of Ashkenazi Jewish descent, developed dystonia of his right and then left arm at the age

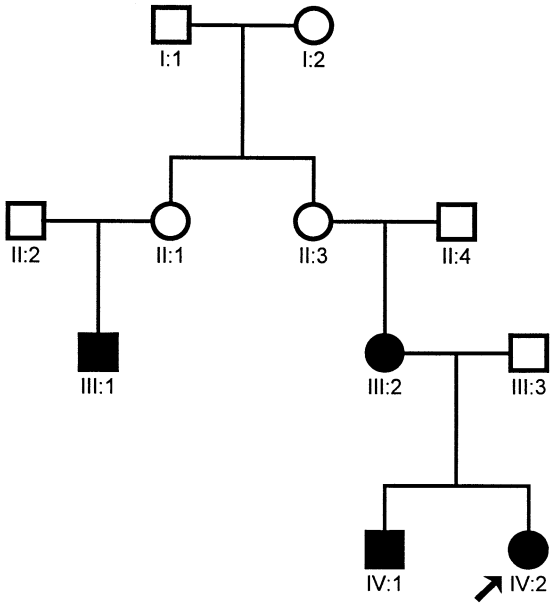

Fig. 3 Pedigree of Family W.

of 10 years. He worsened for a year or two after onset and then had no further progression. When he was seen at the age of 35 years, he had segmental dystonia affecting his arms alone, with no involvement of his legs or craniocervical region. His father also developed dystonia of his arms when he was 8 years old. On follow-up at 30 years of age he still only had segmental dystonia of his arms and has had no further progression since.

Mutation-negative cases

One-hundred and twenty-eight patients were negative for the GAG deletion in the DYT1 gene. Forty-eight patients (38%) were sporadic cases, while 80 (62%) had at least one relative affected by PTD. Four were Ashkenazi Jewish, one was Sephardic Jewish and one had a single parent of Ashkenazi Jewish origin. The age at onset varied widely (mean 35.0 years, SD 17.7 years, minimum 2 years, maximum 74 years).

One-hundred and five of the 128 patients (82%) had focal (59 cases) or segmental (46 cases) dystonia. The age at onset in this group was <28 years in 26 patients and >28 years in 79 patients (mean 38.3 years, SD 25.1 years, minimum 4 years, maximum 74 years).

Twenty-three out of 128 mutation-negative patients (18%) presented with generalized dystonia; in 13 of them, dystonia involved the limbs and the craniocervical muscles, while 10 patients presented with early, limb-onset dystonia without craniocervical involvement (typical DYT1 phenotype). The age at onset in this group was <28 years in all but two patients (mean 13.1 years; SD 8.1 years; minimum 3 years; maximum 33 years).

Discussion

This study provides evidence that the GAG deletion in the DYT1 gene is responsible for the majority of cases of early, limb-onset dystonia in both Jewish and non-Jewish European families. These results are consistent with the findings of Ozelius *et al*. (1997) in North American PTD families, and support the contention that a single mutation is responsible for the majority of cases with typical early-onset dystonia, regardless of the ethnic background and the ancestral origin of the patients. The clinical subgroups used in this work differ slightly from those used by Ozelius *et al*. (1997). We decided to use a simple clinical classification, i.e. generalized, segmental and focal dystonia groups, rather than the broad categories ‘typical’, ‘atypical’ and ‘uncertain’. We maintained the term ‘typical DYT1 phenotype’ only for the selected group characterized by early limb-onset generalized dystonia without spread to the craniocervical muscles. These criteria allow a simpler and more direct comparison between genotype and phenotype in dystonia. Furthermore, if we apply Ozelius’s classification system to our data, the percentage of DYT1-positive cases with ‘typical or probable phenotype’ is 61%, thus very similar to the figure obtained using our classification.

We found the DYT1 mutation in 60% (15/25) of patients with the typical DYT1 phenotype, the majority of whom were from small non-Jewish families of unknown linkage. Only three of these were Ashkenazi Jewish and all three carried the $DYT1^{AJ}$ haplotype. In the USA 74% of individuals with this phenotype carried the deletion. This may reflect the fact that the European population sampled in this study is more heterogeneous than the population of North America, or that it contains a smaller number of Ashkenazi patients. It should be noted that the majority of our cases were recruited through an epidemiological study and therefore may well represent a more accurate estimate of the true proportion of DYT1 positive cases. Our study also confirms the data reported by Almasy *et al*. (1997), who found significant differences in age at onset and site at onset of PTD among different ethnic groups. The high proportion of European PTD patients with typical DYT1 phenotype who do not carry the mutation (40%) may represent either allelic or locus heterogeneity in dystonia. Further work is required to sequence the DYT1 gene in such cases to try and identify other mutations.

This study broadens the phenotype associated with the GAG deletion in the DYT1 gene. We found this mutation in four patients affected by generalized dystonia with craniocervical involvement, two patients with segmental dystonia and one patient with focal right arm dystonia. We screened the available affected relatives of these patients (five subjects). One of them presented with a typical DYT1 phenotype and another with the DYT1 phenotype plus laryngeal involvement. The other three subjects had a milder clinical picture (focal dystonia of the arm or neck or segmental craniocervical dystonia). All these five subjects carried the DYT1 mutation. Family W demonstrates that there is phenotypic variation within a single kindred. Whether this represents variable expression of the GAG mutation or the effect of modifying genes or environmental factors is unclear.

For the majority of cases of focal dystonia, however, the GAG deletion does not appear to be the pathogenic mutation. Ozelius found no deletion carriers among patients with focal or segmental craniocervical dystonia. This may be due to the fact that other genetic loci are involved or that the cases are not genetic. It is interesting to note that Bressman *et al*. (1994) reported several cases of Ashkenazi Jews with segmental or focal dystonia carrying the Ashkenazi DYT1 haplotype, implying they should have the GAG deletion.

This study highlights the importance of the GAG deletion in the DYT1 gene in cases of early, limb-onset generalized dystonia. The condition is of sufficient severity that testing for this mutation may be warranted given appropriate genetic counselling. More importantly, the discovery of the gene causing generalized dystonia will lead to functional studies to understand how this gene can cause the sustained involuntary muscle contractions.

Acknowledgements

This research was partly supported by Comitato Promotore Telethon. E.M.V. is the recipient of a research grant by Consiglio Nazionale delle Ricerche, Italy.

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Received May 21, 1998. Revised July 16, 1998.
Accepted August 17, 1998