

Italian Family With Cranial Cervical Dystonia: Clinical and Genetic Study

*Emanuele Cassetta, MD, †Nicoletta Del Grosso, MSc, *Anna Rita Bentivoglio, MD, PhD,
*‡Enza Maria Valente, MD, †Marina Frontali, MD, and *||Alberto Albanese, MD

**Istituto di Neurologia, Università Cattolica del Sacro Cuore, Rome; †Consiglio Nazionale delle Ricerche, Rome, Italy;
‡University Department of Clinical Neurology, Institute of Neurology, London; and the ||Service de Neurologie, Université de
Lausanne, Lausanne, Switzerland*

Summary: A white Italian family affected by primary torsion dystonia (PTD) is described. The family phenotype most commonly presented with adult onset, cranial cervical involvement, and focal or segmental distribution without progression to generalization. Thirty-nine family members and nine spouses were studied. Five subjects received a diagnosis of definite PTD, three of probable PTD. Age at onset was in adulthood for all. In four definitely affected subjects, dystonia started in the cranial or cervical districts; in one it presented as writer's cramp. Familial writer's cramp also occurred in the family of the unrelated parent of the latter patient. The mean age at time of

examination was 61.8 years in the individuals with a definite diagnosis; 60 in those with a probable diagnosis. At the time of examination, in most of the affected subjects, dystonia was focal; in three cases (two definitely and one probably affected), it was segmental. DNA linkage analysis, although limited by the size of the family, suggested exclusion of linkage between the disease and known PTD loci (DYT6 and DYT7). The GAG deletion in the DYT1 gene was excluded in the proband and in the family member affected by writer's cramp. **Key Words:** Dystonia—Genetics—Family studies—Phenotype—Linkage—DYT1—DYT6—DYT7.

Cervical dystonia is the most common presentation of primary torsion dystonia (PTD) and often occurs in familial aggregation. Families presenting with isolated cervical dystonia are rare. In a large German family with nonprogressive adult-onset cervical or laryngeal dystonia, linkage analysis assigned a PTD gene (DYT7) to the telomeric region of chromosome 18p.¹ Another genetic locus for cervical dystonia has been mapped to chromosome 8 (DYT6) in two non-Jewish German Mennonite families with a phenotype characterized by variability of age at onset and tendency to progression of dystonia.²

We observed an Italian family affected by PTD with clinical features similar to those of the German family linked to DYT7.

MATERIALS AND METHODS

Clinical Study

This family was ascertained through an index case seen at the Movement Disorders Clinic of the Gemelli Hospital of Rome. It is family 2 in the Gemelli Dystonia Registry. The index case was admitted and studied according to a standard protocol for dystonia.³

Participating family members older than 18 years signed an informed consent. Minors were not included in the study for ethical reasons. Two neurologists (EC, ARB) performed all on-site interviews and evaluations. All the examined subjects provided information on familial and personal medical history and checked a detailed questionnaire of common motor problems observed in movement disorders. The neurologic evaluation included specific tasks for dystonia, tremor, or other movement disorders. Blink rate was assessed in all subjects according to published guidelines and compared with values expected.⁴ Each subject was videotaped during the assessment. On-site examiners established a diagnosis of definite or probable dystonia and identified

Received August 4, 1998; revision received February 5, 1999. Accepted May 28, 1999.

Address correspondence and reprint requests to Alberto Albanese, MD, Service de Neurologie, CHUV, Rue du Bugnon, CH-1011 Lausanne, Switzerland.

the body segments involved. A senior neurologist (AA) reviewed the videotapes blinded on the presumptive diagnosis and on the family history. A definite diagnosis was established only in case of unanimity of the three examiners.

A definite diagnosis of blepharospasm was established when all the examiners observed at least two prolonged dystonic spasms of the orbicularis oculi muscle (or of any other periocular muscles) during the whole duration of videotape (approximately 10 minutes). Subjects were considered probably affected by blepharospasm when their blink rate was increased, as compared with the blink rate of the normal population,⁴ or when overactivity of the eyelids but no prolonged spasms were observed. Definite cervical dystonia was characterized by slow dystonic movements or abnormal neck postures associated with jerky movements of the head. When just jerky movements or abnormal postures were observed, the diagnosis was considered probable. A definite diagnosis of upper limb dystonia was established when slow dystonic movements and definitely abnormal postures occurred at rest or were activated and were progressively aggravated by specific tasks. Subjects with jerky, irregular, asymmetric tremor evident during the nose-finger maneuver or mildly abnormal postures were considered probably affected. Scoliosis, mirroring, or clumsiness of the limbs was annotated but was not considered diagnostic for dystonia.

Molecular Genetic Analysis

DNA was extracted from peripheral blood leukocytes using standard techniques. The following microsatellite markers spanning the critical regions on chromosomes 8 (DYT6) and 18 (DYT7) were analyzed:

- DYT6: *cen*—D8S1791—8.3cM—D8S285—8.2cM—D8S260—11.4cM—D8S279—3.3cM—D8S286—*tel*
- DYT7: *cen*—D18S54—10cM—D18S62—0cM—D18S452—14.7cM—D18S464—2.3cM—D18S1153—1.6cM—D18S1150—*tel*

Microsatellite markers were amplified from genomic DNA using the polymerase chain reaction and analyzed as previously described.³

Pairwise linkage analysis was performed using the MLINK option of the FASTLINK program, assuming autosomal-dominant inheritance with 40% penetrance, and a disease gene frequency of 0.0001. The affected identical twins were considered as a single person for the purpose of linkage. Subject IV:27, who was affected by definite upper limb dystonia, was not considered for linkage analysis because his father (III:27), unrelated to this family, belonged to a family affected by writer's cramp (family 23 of the Gemelli registry).

The detection of the DYT1 mutation⁵ was performed as described.⁶

RESULTS

Figure 1 shows the combined pedigrees of family 2 (on the left side) and family 23 (on the right side). Subjects IV:27 and IV:28 belong to family 2 from the maternal side and to family 23 from the paternal side.

We examined 48 subjects in family 2, including 39 family members and nine spouses. Twenty subjects belonged to the third generation, 23 to the fourth, and three to the fifth. Among the 39 family members studied, eight had a diagnosis of dystonia (either definite or probable) and 31 were unaffected. The latter were 15 men and 16 women with a mean age ($\pm$ standard deviation [SD]) of 52.7 ± 15.4 years (range: 26–80 yrs). All of the definitely or probably affected members belonged to the third or fourth generation. Only three patients descended from subject II:2 who was the only member of the second generation reportedly affected by dystonia. Subject II:6, who was the spouse of subject II:5 and the sister of subject II:1, was reportedly affected by head tremor compatible with the diagnosis of essential tremor. The pedigree of family 2 indicated an autosomal-dominant pattern of inheritance with reduced penetrance. Table 1 summarizes the clinical data for family 2.

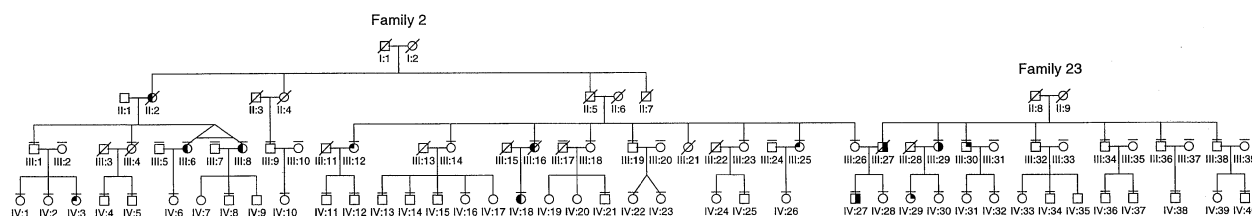


FIG. 1. Combined pedigrees of families 2 and 23. Cranial cervical dystonia is indicated by filling of the left half (definitely affected) or of the upper left quadrant (possibly affected). Upper limb dystonia is indicated by filling of the right half (definitely affected) or of the upper right quadrant (possibly affected). Examined subjects are indicated by a horizontal tract.

TABLE 1. Clinical data

Subject	PTD	At onset		At time of evaluation	
		Age	Phenotype	Age	Phenotype
Definite cases					
III:6 (F)	Segmental	65	Retrocollis	69	Cranial-cervical (face, neck, larynx), trunk, upper limbs
III:8 (F)	Segmental	66	Cranial (peri-oral musculature)	69	Cranial-cervical (face and neck)
III:16 (F)	Focal			74	Cranial (upper and lower face muscles)
IV:18 (F)	Focal	Early adulthood	Blepharospasm	54	Cranial (blepharospasm)
IV:29 (M)	Focal	40	Writer's cramp	43	Right upper limb (writer's cramp)
Probable cases					
III:12 (F)	Segmental			80	Blepharospasm, lower face muscles, dystonia, laterocollis
III:27 (F)	Focal			60	Blepharospasm
IV:3 (F)	Focal			56	Blepharospasm

PTD, primary torsion dystonia.

Definite Dystonia

Five subjects (four women and one man) received a diagnosis of definite PTD. Two patients (III:6 and III:8) were identical twin sisters; the remaining two women were first-degree relatives (subject III:16 and her daughter). The mean age ($\pm$ SD) at the time of study was 61.8 ± 12.9 years (range: 43–74 yrs). The age at disease onset (clearly identified only for cases III:6, III:8, and IV:27) was 65, 66, and 40 years, respectively. The interval between disease onset and the first clinical examination was 4 years in one case; 3 years for the two other cases. Dystonia started in the cranial district in three subjects, in the cervical region in one, and in one upper limb in one. Progression to other body regions was observed only in two subjects. At the time of examination, the clinical presentation of dystonia was segmental in two patients and focal in three (two cases of cranial dystonia and one case of upper limb dystonia).

Subject III:6 (Segmental Dystonia)

This 69-year-old woman was the index case of the family. She was born after an uncomplicated full-term twin pregnancy; no problems occurred during delivery. At the age of 65, she noted the onset of retrocollis. Dystonia rapidly progressed to cranial muscles, to the platysma, larynx, trunk, and eventually to the upper limbs within 1 year from onset. On examination, blepharospasm, spasmodic dysphonia, facial, cervical, upper limb, and trunk dystonia were the prominent signs. Blink rate was higher than expected: 84 blinks per minute during conversation, 80 at rest, and 6 while reading. Video laryngoscopic evaluation confirmed the diagnosis of laryngeal dystonia. She had no pyramidal, cerebellar, or sensory signs, and her intellect was normal. No clinical features of secondary dystonia, paroxysmal dystonia, or alcohol-responsive myoclonic dystonia were observed. Brain computed tomography (CT) and magnetic reso-

nance imaging were unremarkable. Biochemical routine for secondary dystonia was negative. The patient received treatment with 250/25 mg levodopa/carbidopa four times a day for 8 weeks without clinical improvement. Acute ingestion of 30 mL of alcohol failed to ameliorate dystonia. She was treated with different combinations of psychoactive drugs, myorelaxants, and anticholinergics in association with botulinum toxin, which improved blepharospasm and cervical dystonia. At variance with her twin sister, this woman used to carry heavy weights on her head for many years before the onset of cervical dystonia.

Subject III:8 (Segmental Dystonia)

She was the homozygotic twin sister of the index case. Early development was normal. At the age of 66, she first had involuntary movements of the perioral muscles. Later, dystonia progressed to other cranial muscles and to the neck. Brain CT scan and biochemical laboratory tests were unremarkable. There were no identifiable causes of secondary dystonia and no precipitating factors. At the time of the first examination, she had blepharospasm and cranial dystonia, involuntary contractions of the platysma, a slight bilateral tremor of the upper limbs, and a tremulous voice. Blink rate was 80 during conversation, 54 at rest, and 24 while reading.

Subject III:16 (Focal Dystonia)

This 74-year-old woman did not have any symptoms. Her relatives had noticed abnormal movements of the periocular facial muscles during the last few years. On examination, involuntary movements of the lower eyelids and spasmodic contractions of the orbicularis oculi (matching the stated criteria for definite blepharospasm) were observed. Voice tremor and "yes-yes" tremor of the head were also observed. Prolonged spasms occurred during conversation; blink rate was within the normal

range: 19 during conversation, 22 at rest, and 2 while reading; however, at variance with the pattern commonly observed in the normal population,⁴ blink rate at rest was higher than during conversation.

Subject IV:18 (Focal Dystonia)

At the time of clinical evaluation, this woman was 54 and unaware of having dystonia. She reported increased blink rate as a consequence of emotional states and ocular discomfort when exposed to bright light. She could not recall when this trouble started; other family members reported that her blinking was exaggerated since she was a young girl. On examination, she had blepharospasm, involuntary movements of the lips and jaw, and a mild bilateral postural tremor of the upper limbs. Blink rate was slightly higher than normal during conversation (60 blinks per minute) and while reading¹¹ but normal at rest.²⁶

Subject IV:27 (Focal Dystonia)

This 43-year-old right-handed bank employee was born after an uncomplicated pregnancy and a normal delivery. Personal history was uneventful. Since adolescence, he presented with bilateral hand tremor and eye discomfort in bright light. He had difficulties in writing for the last 3 years. Approximately 1 year before examination, he had to stop writing with his right hand and start typing because of the increasing pain and the illegibility of his handwriting. For this reason, he required medical intervention. On neurologic examination, abnormalities during handwriting were observed: writing with the right hand required effort; forearm muscles were abnormally tense and contracted, although the quality of handwriting did not worsen progressively; and hand grip was abnormal. Blink rate was normal: 32 during conversation, 19 at rest, and 6 while reading. The patient's father, unrelated to family 2, was reportedly affected by bilateral hand tremor and presented in late adulthood with abnormalities in his handwriting suggestive of writer's cramp. An evaluation of the paternal family of this patient (family 23) revealed familial dystonia (one case of definite and two of probable upper limb dystonia). The DYT1 mutation was excluded in this subject.

Probable Dystonia

Three women received a diagnosis of probable dystonia. Their mean age at the time of the study was 65.3 ± 12.9 years (range 56–80 yrs). Age at disease onset could not be ascertained.

Subject III:12 (Segmental Dystonia)

This 80-year-old woman did not have any symptoms. In 1985 she was diagnosed with depression and was

treated with nortriptyline. On observation, overactivity of the lower eyelid was evident. Blink rate was 27 during conversation, 3 at rest; it was impossible to evaluate blink rate while reading because of a severe visual impairment. A mild laterocollis and occasional lip and jaw protrusion were observed. Contralateral mirror movements occurred bilaterally when performing repetitive movements of the lower limbs.

Subject III:25 (Focal Dystonia)

This 56-year-old patient reported frequent and intermittent involuntary movements of the lips and tongue, rigidity of the neck, clumsiness in drawing, and eye discomfort (sensation of dry eyes). On examination, frequent eye blinking, involuntary lip protrusion, some involuntary movements of upper and lower facial muscles and of the platysma were evident. Blink rate was normal: 60 during conversation, 72 at rest, and 14 while reading.

Subject IV:3 (Focal Dystonia)

This 60-year-old woman reported eye discomfort when exposed to bright light and head tremor. On examination, her blink rate was higher than expected: 46 during conversation, 78 at rest, and 6 while reading. The relative blink rate pattern was also abnormal. A mild head tremor and clumsy movements of the left lower limb were observed.

Second and Fifth Generations

No family members of the second generation were alive at the time of the study. Subject II:2 was reportedly affected by blepharospasm. No signs of dystonia were reported in subject II:5. Subject II:6 was reported as affected by a mild tremor of the head. All the examined subjects belonging to the fifth generation (the two offspring of subject IV:18 and the son of subject IV:11) were unaffected by dystonia.

Molecular Genetic Study

Markers D8S1791 and D18S1150 were not informative in this family and were excluded from linkage analysis. Subjects with probable dystonia were classified either as "unknown phenotype" (condition A) or as "affected" (condition B). Pairwise linkage analysis between dystonia and the informative markers spanning the DYT6 and DYT7 areas produced negative lod scores under both conditions. Table 2 shows two-point lod scores between the disease locus and the typed markers under condition A. More negative values were obtained under condition B (data not shown). The haplotype reconstruction for both DYT6 and DYT7 areas failed to show a common haplotype shared by all the affected

TABLE 2. Two-point lod scores for PTD and chromosome 8 and 18 markers under condition A

Lod score at Θ		0.0	0.01	0.05	0.1	0.2	0.3	0.4
Chromosome 8	D8S285	-3.22	-1.10	-0.44	-0.20	-0.03	+0.01	+0.01
	D8S260	-3.21	-1.23	-0.58	-0.33	-0.13	-0.04	0.00
	D8S279	-4.16	-2.23	-1.18	-0.70	-0.29	-0.11	-0.02
	D8S286	-3.40	-1.50	-0.82	-0.53	-0.26	-0.11	-0.03
Chromosome 18	D18S54	-3.43	-1.56	-0.80	-0.46	-0.15	-0.03	+0.01
	D18S62	-3.53	-1.54	-0.80	-0.47	-0.18	-0.06	-0.02
	D18S452	-3.69	-1.47	-0.76	-0.45	-0.17	-0.05	-0.01
	D18S464	-0.28	-0.27	-0.24	-0.19	-0.11	-0.05	-0.01
	D18S1153	-0.73	-0.65	-0.40	-0.20	-0.11	-0.06	0.00

PTD, primary torsion dystonia.

members. The 3-bp deletion in the DYT1 gene was not detected in the index case (subject III:6).

DISCUSSION

This Italian family presented with a homogeneous phenotype characterized by cranial cervical dystonia. Clinical presentation was so remarkably homogeneous that the observation of subject IV:27 with upper limb dystonia prompted us to study his paternal family, which was indeed affected by familial writer's cramp. The clinical presentation of dystonia in subject IV:27 actually suggests that he may have inherited writer's cramp from his father.

The structure of family 2 was poorly informative. Of five definitely affected subjects, two were monozygotic twins, thus sharing the same DNA information, and one possibly inherited writer's cramp from the unrelated parent. Linkage analysis was therefore carried out on three definitely affected and three probably affected subjects.

The onset and progression of dystonia were different in the index case and in her monozygotic twin. The index

case, which was the only subject in the family who used to carry heavy weights on her head, had a clinical picture noticeably worse than that of the remaining affected subjects. It appears plausible that this chronic environmental factor, lasting for years and directly affecting the cervical region, could be responsible for repeated strain and for a more severe phenotypic expression of dystonia.⁷

Mild dystonia is difficult to ascertain in families with a focal dystonia phenotype. Two patients in family 2 were unaware of being affected because of their mild clinical course. Underestimation of dystonia cases may reduce the power of genetic analysis, whereas overestimation may yield false results. In the present study we applied strict clinical criteria and blind videotape revision as a means to reach a reliable consensus. Analysis of blink rate patterns⁴ was not used as a diagnostic criterion, because its clinical value for the diagnosis of dystonia is still under scrutiny.

The 3-bp deletion in the DYT1 gene was not detected in the index case, the most severely affected family member. Also, the clinical presentation of dystonia in this family differed noticeably from the typical DYT1 phenotype (early limb-onset dystonia without spread to the craniocervical region).^{1,2,6} Taken together, these data make it unlikely that the responsible gene for dystonia in this family could be DYT1, although a different, still unmapped gene mutation cannot be ruled out.

Linkage of the disease to DYT6 and DYT7 genes cannot be formally excluded because the limited number of affected members and the low penetrance of the disease restrict linkage power in the family. Pairwise linkage analysis, however, showed negative lod scores throughout the areas, considering the subjects with probable dystonia either as unknown phenotype (condition A) or as affected (condition B). Furthermore, the affected members of the family did not share the same haplotype for the DYT6 or DYT7 regions. These results suggest that dystonia in this family is probably linked to a still unmapped gene.

TABLE 3. Clinical and genetic data of this family and of the German family linked to DYT7

	Present family	DYT7-linked family (1)
Linkage	Unknown	DYT7 (18p)
Definitely affected subjects	4 + 1*	7
Age at onset (yrs)	Adult (40–66)	Adult (28–70)
Phenotype at onset	• Cranial: 3 cases • Cervical: 1 case • Upper limb: 1 case*	• Cervical: 6 cases • Laryngeal: 1 case
Progression (no. of cases)	• Multisegmental (1) • Cranial-cervical (1) • Blepharospasm (2) • Writer's cramp (1)*	• Cervical (6†) • Dysphonia (1)

* This patient had a positive family history either from the maternal side (present family) or from the paternal side (familial writer's cramp).

† Among them, one case of writer's cramp and one of Meige's syndrome were also diagnosed.

Families with cervical dystonia have already been described. The family described here clearly resembles the German family linked to DYT7 in which six of seven definitely affected members presented with adult-onset, focal cervical dystonia.¹ Table 3 compares phenotypic features of these two families. Conversely, this family appears phenotypically different from another Italian family that we have observed³ and from the two German Mennonite families linked to the DYT6 gene.²

This family provides further evidence that PTD is clinically and genetically heterogeneous and supports the hypothesis that additional loci are responsible for the adult-onset form of PTD. More extensive data could be collected if families with similar clinical features are pooled for the purpose of molecular genetic studies.

Acknowledgments: Financial support from Fondazione Telethon (grant E.499) is acknowledged. Dr. EM Valente is the recipient of a grant from Consiglio Nazionale delle Ricerche, Italy.

REFERENCES

1. Leube B, Rudnicki D, Ratzlaff T, Kessler KR, Benecke R, Auburger G. 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;5:1673–1677.
2. Almasy L, Bressman SB, Raymond D, et al. Idiopathic torsion dystonia linked to chromosome 8 in two Mennonite families. *Ann Neurol* 1997;42:670–673.
3. Bentivoglio AR, Del Grosso N, Albanese A, Cassetta E, Tonali P, Frontali M. Non-DYT1 dystonia in a large Italian family. *J Neurol Neurosurg Psychiatry* 1997;62:357–360.
4. Bentivoglio AR, Bressman SB, Cassetta E, Carretta D, Tonali P, Albanese A. Analysis of blink rate patterns in normal subjects. *Mov Disord* 1997;12:1028–1034.
5. Ozelius LJ, Hewett JW, Page CE, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. *Nat Genet* 1997;17:40–48.
6. Valente E, Warner T, Jarman P, et al. The role of DYT1 in primary torsion dystonia in Europe. *Brain* 1998;121:2335–2339.
7. Bentivoglio AR, Cassetta E, Del Grosso N, Albanese A, Frontali M, Tonali P. Different phenotypic features of dystonia in monozygotic twin sisters belonging to a large family affected by idiopathic torsion dystonia (ITD). *European Journal of Neurology* 1997;4:S2–S3.