

DYT13, a Novel Primary Torsion Dystonia Locus, Maps to Chromosome 1p36.13–36.32 in an Italian Family with Cranial-Cervical or Upper Limb Onset

Enza Maria Valente, MD,¹ Anna Rita Bentivoglio, MD, PhD,² Emanuele Cassetta, MD,^{2,3} Peter H. Dixon, PhD,¹ Mary B. Davis, PhD,¹ Alessandro Ferraris, MD,² Tamara Ialongo, MD,² Marina Frontali, MD,⁴ Nicholas W. Wood, PhD, FRCP,² and Alberto Albanese, MD^{1,5}

Primary torsion dystonia (PTD) is a clinically and genetically heterogeneous group of movement disorders, usually inherited in an autosomal dominant fashion with reduced penetrance. The DYT1 gene on chromosome 9q34 is responsible for most cases of early limb-onset PTD. Two other PTD loci have been mapped to date. The DYT6 locus on chromosome 8 is associated with a mixed phenotype, whereas the DYT7 locus on chromosome 18p is associated with adult onset focal cervical dystonia. Several families have been described in which linkage to the known PTD loci have been excluded. We identified a large Italian PTD family with 11 definitely affected members. Phenotype was characterized by prominent cranial-cervical and upper limb involvement and mild severity. A genome-wide search was performed in the family. Linkage analysis and haplotype construction allowed us to identify a novel PTD locus (DYT13) within a 22 cM interval on the short arm of chromosome 1, with a maximum lod score of 3.44 between the disease and marker D1S2667.

Ann Neurol 2001;49:362–366

Dystonia is characterised by sustained involuntary muscle contractions causing twisting movements and abnormal postures, without other neurological signs.¹ Primary torsion dystonia (PTD) is a movement disorder in which dystonia is the primary and indeed sole abnormality directly attributable to the condition.² PTD has a wide clinical spectrum and may be generalized, segmental or focal, its severity being largely determined by the age of onset. Patients with onset in childhood tend to develop severe generalized dystonia, whereas onset in adult life (commonly in cranial or cervical muscles) is less frequently associated to spreading to other body districts and to generalization.³ PTD is often inherited in an autosomal dominant pattern with reduced penetrance (30–40%).² Three PTD loci have been mapped to date. The gene responsible for early limb-onset generalized dystonia (DYT1) has been linked to chromosome 9q34⁴ and has recently been cloned. The only detected mutation is a three-base pair (GAG) deletion, resulting in loss of a glutamic-

-acid residue in a conserved region of a novel adenosine triphosphate (ATP)-binding protein, termed TorsinA.^{5,6} A form of adult-onset, focal PTD (DYT7) has been linked to chromosome 18p in a German family,⁷ whereas in two German-Mennonite families showing a mixed phenotype a novel locus (DYT6) has been mapped to chromosome 8.⁸ Linkage to known chromosomal locations has been excluded in several PTD families, in which other genes are likely to be involved.^{9–14} The identification of novel PTD genes is particularly difficult, as families with dystonia are often too small for linkage purposes and the heterogeneity of clinical presentation does not allow pooling of families.

The large Italian PTD family described here was considered highly informative and a suitable resource to identify a novel PTD gene. We performed a genome-wide search and mapped a novel locus, named DYT13, on chromosome 1p36.13–36.32, to a 22cM region with high gene density.

From the ¹Department of Clinical Neurology, Institute of Neurology, London, United Kingdom; the ²Department of Neurology, Catholic University, Rome; the ³AFaR Fatebenefratelli Hospital, Rome; the ⁴Institute of Experimental Medicine, CNR, Rome; and the ⁵National Neurological Institute "Carlo Besta," Milan, Italy.

Received Jun 12, 2000, and in revised form Sep 26. Accepted for publication Sep 26, 2000.

Address correspondence to Dr Wood, Neurogenetics, Department of Clinical Neurology, Institute of Neurology, Queen Square, London WC1N 3BG, UK. E-mail: n.wood@ion.ucl.ac.uk

Subjects and Methods

Subjects

A large Italian family composed of 45 family members and 11 spouses was investigated; the methodology and the clinical characterization of the family have been reported elsewhere.¹¹ After obtaining informed consent, venous blood samples were taken from all examined subjects for DNA analysis. Family members have been followed up (last examination in March 2000).

DNA and Linkage Analysis

We extracted DNA from leukocytes using standard techniques. Exclusion of linkage between the disease and the already known PTD loci (DYT1, DYT6, and DYT7) has been reported elsewhere.^{11,13} A simulation study performed with the program SLINK¹⁵ revealed a maximum expected lod score of 3.56 at recombination fraction (θ) = 0.00. The family was then considered suitable for a genome-wide analysis. We analyzed 400 highly polymorphic fluorescent microsatellite markers spanning the 22 autosomes with an average distance of 10 cM (Linkage Mapping Set version 2; PE Applied Biosystems, Foster City, CA). All available family members were genotyped to allow haplotype construction and the reconstruction of deceased gene carrier haplotypes with a maximum certainty. Microsatellite markers were amplified from genomic DNA using the polymerase chain reaction (PCR) technique as specified by the manufacturers,

and electrophoresed on a denaturing acrylamide gel using a 377 DNA Sequencer (PE Applied Biosystems). DNA fragment size analysis was performed semiautomatically using the Genescan and Genotyper software (PE Applied Biosystems) to determine genotypes.

Two-point lod scores were generated using the FASTLINK version of the MLINK program,^{16,17} using an assumption of equal male–female recombination rate, autosomal dominant inheritance, reduced penetrance (0.40), a gene frequency of 0.0001, and equal allele frequencies for each marker. Family members diagnosed as having undetermined phenotype (see Results) were not included in the linkage analysis.

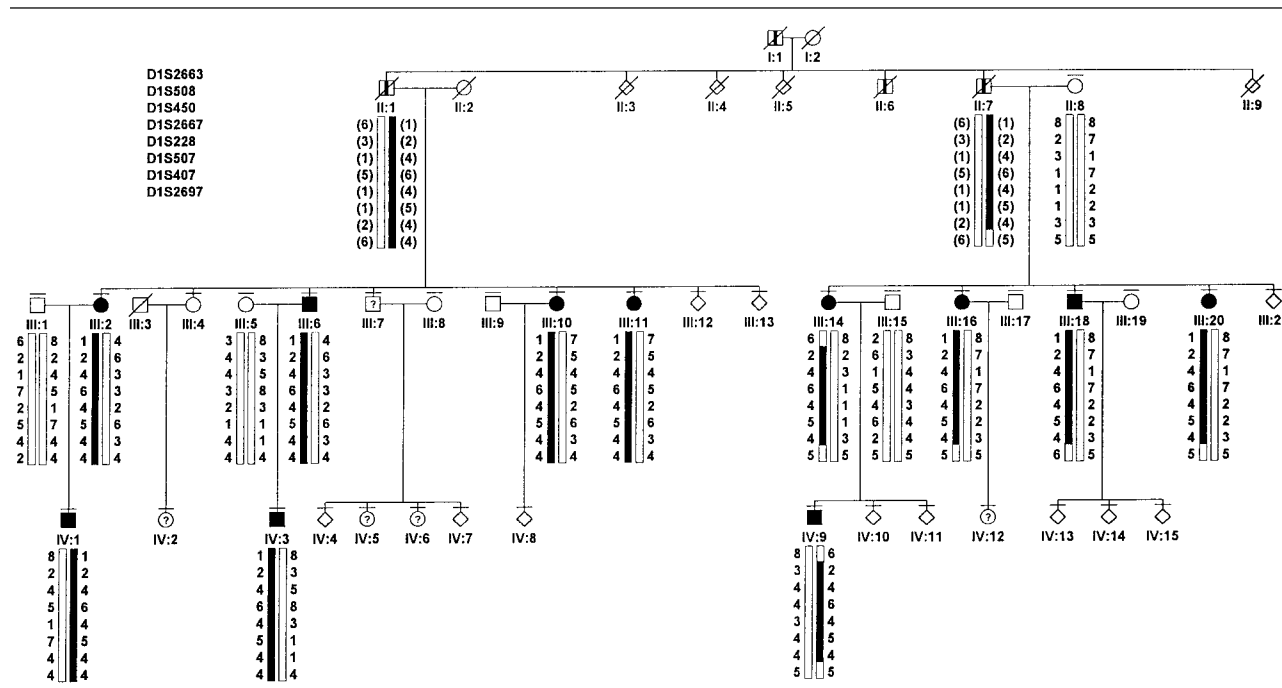
When an lod score ≥ 1 was obtained at a given locus or when linkage between the disease and uninformative markers could be neither proved nor excluded, the surrounding regions were saturated with closely spaced microsatellite markers (average distance 2 cM) and haplotypes were constructed manually. Phase was assigned based on the minimum number of recombinants. Marker order and genetic distances were based on framework markers of the latest Genetic Location Database chromosome 1 consensus map.

Results

Clinical Analysis

A simplified pedigree of the family is shown in the Figure. The family was examined for the first time in

Fig. Simplified pedigree of the family and haplotypes of marker loci spanning the linked region on chromosome 1p36. Black symbols denote individuals affected by PTD, a question mark denotes individuals with undetermined phenotype, and a thick vertical bar within the symbol denotes individuals affected by history. Deceased members are marked with a diagonal bar. A thin horizontal bar above symbols indicates members of the family who were examined clinically. The black bar denotes the haplotype segregating with the disease in the family, whereas any other haplotype is represented by a white bar. To protect patient confidentiality and erroneous conclusions regarding gene status, only the haplotypes of affected family members have been included, and a diamond symbol has been used to mask identity of unaffected individuals.



1994; at that time, eight individuals received a diagnosis of definite dystonia and six a diagnosis of probable dystonia.¹¹ The age at onset in definitely affected subjects ranged from 5 to 40 years. The phenotype was characterized by focal or segmental dystonia with onset either in the cranial-cervical region or in the upper limbs, mild course, and occasional generalization.

All probably affected and unaffected family members agreed to be reevaluated in March 2000. At that time, three more individuals (III:11, IV:9, and III:20) had developed a definite dystonia. Individuals III:11 and IV:9 had been diagnosed as unaffected in 1994. On the latest examination, individual III:11 presented with dystonic tremor and posturing of the neck. Individual IV:9 presented with marked irregular tremor and bilateral dystonic posturing of the upper limbs, and writer's cramp. Individual III:20 had received a diagnosis of probable dystonia in 1994. On the latest examination, she had dystonic posturing of the right arm while writing, and dystonic jerks and posturing of the neck partially controlled by a sensory trick. In these three subjects, aged 55, 41, and 58, respectively, the age at onset could not be accurately defined, as dystonia was mild at onset and worsened slowly over time. The patients or relatives did not take special notice of the symptoms and could not be precise as to date of onset. The remaining five individuals who were diagnosed as probably affected in 1994 did not present evolution over 6 years. They still had minor clinical signs (jerks of neck or of the arm or mild tremor), but no spasmodic movements or postures were evident, and no directional or task-activated movements or sensory tricks. These people have been considered in this study as "undetermined phenotype" and were not included in the linkage analysis.

The inheritance of PTD was autosomal dominant, with affected individuals spanning three consecutive generations and male-to-male transmission. A summary

of the clinical presentation of dystonia in the family is given in Table 1.

Linkage Analysis

Linkage to DYT1, DYT6, and DYT7 had been previously excluded.^{11,13} Four hundred microsatellite markers covering all autosomes were analyzed in the family. All of them generated negative or nonsignificant lod scores at all tested recombination fractions ($\theta = 0.0$ to 0.5), except five markers on chromosomes 1, 5, 10, 12, and 15, which generated maximum lod scores between 1.0 and 1.8. The regions surrounding these five loci and all regions surrounding noninformative markers were then saturated with closely spaced microsatellite markers and haplotypes were constructed. The negative lod scores obtained and the detection of different haplotypes carried by the affected individuals in the family allowed exclusion of all autosomes except a region on the short arm of chromosome 1. All markers spanning this candidate interval produced positive lod scores, with a maximum lod score of 3.44 ($\theta = 0.0$) between the disease and marker D1S2667 (Table 2). Calculation of pairwise lod scores assuming different penetrance values (0.20 to 0.80) and under the assumption "affected individuals only" did not result in a significant change (data not shown). All affected individuals in the family shared a common haplotype between D1S2663 and D1S2697 (see Fig), allowing the identification of a 22 cM interval containing a novel PTD gene (DYT13). The upper extent of the region is determined by recombinations detected in subjects III:14 and IV:9 between markers D1S2663 and D1S450. The lower extent of the region is defined in individual II:7 and his descendants between D1S407 and D1S2697.

Table 1. Clinical Presentation of Dystonia in Definitely Affected Individuals (n = 11)

Subject (Sex)	Onset		Latest Examination	
	Age	Site	Age	PTD Distribution
III:2 (F)	5	Cranial-cervical	71	Upper face, larynx, neck, upper limbs (segmental)
III:6 (M)	10	Cervical	67	Upper face, neck, upper limbs (segmental)
III:10 (F)	26	Cranial-cervical	63	Upper face, larynx, pharynx, neck, upper limbs (segmental)
III:11 (F)	Unknown	Cervical	65	Neck (focal)
III:14 (F)	5	Upper limbs	61	Upper face, larynx, neck, trunk, limbs (generalized)
III:16 (F)	5	Cervical	59	Upper and lower face, neck, trunk (segmental)
III:18 (M)	20	Cervical	56	Lower face, neck, limbs (generalized)
III:20 (F)	Unknown	Cervical	58	Neck, right upper limb (segmental)
IV:1 (M)	40	Right upper limb	45	Right upper limb (focal)
IV:3 (M)	14	Cranial-cervical	32	Lower face, larynx, neck (segmental)
IV:9 (M)	Unknown	Upper limbs	41	Upper limbs (segmental)

F = female; M = male.

Table 2. Pairwise lod Scores between PTD and Markers on Chromosome 1p36

Markers	Intermarker Distance	lod Scores at $\theta =$						
		0.0	0.01	0.05	0.1	0.2	0.3	0.4
D1S2663	1.8cM	-5.94	1.24	1.76	1.81	1.56	1.10	0.51
D1S450		2.52	2.49	2.36	2.17	1.71	1.15	0.50
D1S508	2.0cM							
D1S2667	4.0cM	2.81	2.76	2.56	2.29	1.70	1.06	0.40
D1S228	4.9cM	3.44	3.39	3.17	2.88	2.23	1.49	0.67
D1S507	4.4cM	3.32	3.27	3.06	2.79	2.17	1.46	0.66
D1S407	0.8cM	3.03	3.00	2.83	2.60	2.05	1.39	0.63
D1S2697	2.5cM	3.05	3.00	2.80	2.54	1.96	1.30	0.57
		-6.97	-2.05	-0.76	-0.29	0.03	0.08	0.04

Discussion

We have identified a fourth locus for primary torsion dystonia, DYT13, on the short arm of chromosome 1 in a non-Jewish family from central Italy. The phenotype is characterized by prominent involvement of the cranial-cervical region and the upper limbs; age of onset is variable; progression is mild and disease course is relatively benign with occasional tendency to generalization. All affected individuals, including those with generalized dystonia, were able to accomplish common domestic chores and perform daily living activities.

Nineteen individuals partially or completely shared the haplotype segregating with the disease; 11 of them (58%) were affected by dystonia. This value of penetrance is slightly higher than the penetrance usually attributed to primary dystonia genes (30–40%)²; however, not all unaffected members of the family were available for clinical examination and genotyping, so the exact value of penetrance for the DYT13 gene remains to be defined.

The clinical picture is noticeably different from the DYT1 phenotype, where dystonia presents generally in a limb, rarely affects the cranial-cervical region, and has a higher tendency to generalize, producing a much more disabling disease.^{5,18} The DYT6-associated phenotype is characterized by a wider distribution of body regions involved at onset and in the course of the disease, which has the tendency to be more severe and to generalize more frequently.⁸ The phenotype in our family is also different from that described for the DYT7 gene, which is characterized by adult-onset pure focal cervical dystonia without tendency to spread to other body regions.⁷

In several PTD families reported so far, linkage to the known PTD loci has been excluded; in some of these families the phenotype shares relevant clinical fea-

tures with DYT13-linked dystonia. In two large non-Jewish families reported in 1996 by Bressman and co-workers (one previously described by Uitti and Maraganore¹⁹), the affected members presented with early or adult-onset dystonia confined to cervical and brachial region.¹⁰ Two other PTD families, of Swedish and Italian origin, had a similar phenotypic presentation: variable age at onset (spanning from the second to the fifth decade), cranial-cervical prominent involvement, and upper limb tremor or occasional generalization.^{14,20} Families whose phenotype is remarkably different from DYT13-linked dystonia have also been reported. A family observed by Parker had a variable phenotypic presentation, characterized by prominent laryngeal involvement, torticollis, and infrequent generalization; Wilson's disease also occurred in the same family.²¹ The underlying dystonia gene in this family was named DYT4, but its chromosomal location has not been established. An Italian family from South Tyrol displayed an unusually variable phenotype: Most affected members had cervical or upper limb dystonia with onset in adulthood, although some patients suffered from typical early-onset generalized dystonia.¹² Some of these families may link to the DYT13 locus, as many of them were characterized by variable age of onset (juvenile or adult) and prominent cranial-cervical involvement.

A large number of genes map within the 22 cM candidate interval identified in our family, but none of them represents an obvious candidate for dystonia. The most interesting gene mapping to the region is a gene coding for a member of the heat-shock protein family, called cvHsp. This protein is mainly expressed in cardiovascular tissues, but a low expression has been also detected in specific areas of the brain, i.e., putamen, caudate, substantia nigra, and amygdala.²² This gene

represents an interesting candidate because TorsinA, the DYT1 gene product, is a protein with high similarities to heat-shock proteins.^{5,6} Other genes map within the linked region and bear a possible role in neurological diseases: SCNN1D, an amiloride-sensitive nonvoltage gated sodium channel, isoform delta, expressed in brain and other tissues, is putatively involved in neurodegeneration.²³ EPHA2, a tyrosine kinase receptor expressed in projecting neurones and their target fields, is involved in short-range, contact-mediated, axonal guidance.²⁴ KCNA2B codes for the $\beta 2$ subunit of a voltage gated "shaker" potassium channel,²⁵ and DVL1, a widely expressed homologue of a *Drosophila* gene, is putatively involved in neural and heart development.²⁶

The role of this novel dystonia locus remains to be tested in other PTD families and in the general population, as most patients affected by cranial-cervical or upper limb (focal or segmental) dystonia have a sporadic occurrence. The identification of other dystonia families linked to chromosome 1p will help refine the locus position on the genetic map, which is an essential step toward identification of the gene and its function.

Dr Valente was partly supported by a CNR grant. Dr Dixon and Dr Wood were supported by MRC program grant G9706148. This project was supported in part by Telethon grant E499.

Electronic database information: Genetic Location Database: <http://cedar.genetics.soton.ac.uk/pub/chrom1/gmap>. GeneMap '99: <http://www.ncbi.nlm.nih.gov/genemap/>

References

1. Fahn S, Marsden CD, Caln DB. Classification and investigation of dystonia. In: Marsden CD, Fahn S, eds. *Movement disorders 2*. London: Butterworths, 1987:332–358.
2. Bressman SB. Dystonia. *Curr Opin Neurol* 1998;11:363–372.
3. Greene P, Kang UJ, Fahn S. Spread of symptoms in idiopathic torsion dystonia. *Mov Disord* 1995;10:143–152.
4. Ozelius LJ, Kramer PL, Moskowitz CB, et al. Human gene for torsion dystonia located on chromosome 9q32–34. *Neuron* 1989;2:1427–1434.
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. Ozelius LJ, Page CE, Klein C, et al. The TOR1A (DYT1) gene family and its role in early onset torsion dystonia. *Genomics* 1999;62:377–384.
7. 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;5:1673–1677.
8. 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.
9. Bressman SB, Heiman GA, Nygaard TG, et al. A study of idiopathic torsion dystonia in a non-Jewish family: evidence for genetic heterogeneity. *Neurology* 1994;44:283–287.
10. Bressman SB, Warner TT, Almasy L, et al. Exclusion of the DYT1 locus in familial torticollis. *Ann Neurol* 1996;40:681–684.
11. Bentivoglio AR, Del Grosso N, Albanese A, et al. Non-DYT1 dystonia in a large Italian family. *J Neurol Neurosurg Psychiatry* 1997;62:357–360.
12. Klein C, Pramstaller PP, Castellan CC, et al. Clinical and genetic evaluation of a family with a mixed dystonia phenotype from South Tyrol. *Ann Neurol* 1998;44:394–398.
13. Jarman PR, Del Grosso N, Valente EM, et al. Primary torsion dystonia: the search for genes is not over. *J Neurol Neurosurg Psychiatry* 1999;67:395–397.
14. Cassetta E, Del Grosso N, Bentivoglio AR, et al. Italian family with cranial-cervical dystonia: clinical and genetic study. *Mov Disord* 1999;14:820–825.
15. Ott J. Computer-simulation methods in human linkage analysis. *Proc Natl Acad Sci USA* 1989;86:4175–4178.
16. Cottingham RW Jr, Indury RM, Schaffer AA. Faster sequential genetic linkage computations. *Am J Hum Genet* 1993;53:252–263.
17. Schaffer AA. Faster linkage analysis computations for pedigrees with loops or unused alleles. *Hum Hered* 1996;46:226–235.
18. Valente EM, Warner TT, Jarman PR, et al. The role of DYT1 in primary torsion dystonia in Europe. *Brain* 1998;121:2335–2339.
19. Uitti RJ, Maraganore DM. Adult onset familial cervical dystonia: report of a family including monozygotic twins. *Mov Disord* 1993;8:489–494.
20. Holmgren G, Ozelius LJ, Forsgren L, et al. Adult onset idiopathic torsion dystonia is excluded from the DYT1 region (9q34) in a Swedish family. *J Neurol Neurosurg Psychiatry* 1995;59:178–181.
21. Parker N. Hereditary whispering dysphonia. *J Neurol Neurosurg Psychiatry* 1985;48:218–224.
22. Krief S, Faivre J-F, Robert P, et al. Identification and characterization of cvHsp. *J Biol Chem* 1999;274:36592–36600.
23. Waldmann R, Bassilana F, Voilley N, et al. Assignment of the human amiloride-sensitive Na⁺ channel delta isoform to chromosome 1p36.3-p36.2. *Genomics* 1996;34:262–263.
24. Sulman EP, Tang XX, Allen C, et al. ECK, a human EPH-related gene, maps to 1p36.1, a common region of alteration in human cancers. *Genomics* 1997;40:371–374.
25. Schultz D, Litt M, Smith L, et al. Localization of two potassium channel β subunit genes, KCNA1B and KCNA2B. *Genomics* 1996;31:389–391.
26. Pizzuti A, Amati F, Calabrese G, et al. cDNA characterization and chromosomal mapping of two human homologues of the *Drosophila* dishevelled polarity gene. *Hum Mol Genet* 1996;5:953–958.