Clinical Features, *DYT1* Mutation Screening and Genotype-Phenotype Correlation in Patients with Dystonia from Iran

Mohammad Taghi Akbari^a Zahra Zand^b Gholam Ali Shahidi^c
Mohammad Hamid^b

^aDepartment of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, ^bMolecular Medicine Division, Biotechnology Research Center, Pasteur Institute of Iran, and ^cDepartment of Neurology, Iran University of Medical Sciences, Hazrat Rasool Hospital, Tehran, Iran

Key Words

Primary dystonia · Clinical features · *DYT1* gene · GAG deletion

Abstract

Objective: To test Iranian patients with primary torsion dystonia to determine the frequency of 904–906 del GAG mutation in the *DYT1* (*TOR1A*) gene and to investigate the genotype-phenotype association for this disease. **Subjects and Methods:** Sixty-three patients with primary dystonia were investigated. DNA was extracted from peripheral blood and these samples were subjected to PCR-sequencing for exon 5 of the *DYT1* gene. **Results:** Of the 63 patients, 10 (15.9%) carried the triplet GAG deletion mutation; this is a high *DYT1*-positive rate in comparison with other populations and the type of dystonia in this positive group was generalized in all except 1. In our patients, limbs were the most severely involved site at the time of onset and in most cases it developed to generalized form. The majority of *DYT1*-positive cases showed higher leg onset (5 patients, 62.5%) in comparison with higher arm onset in negative patients (20 patients, 50%). Also, the progression to generalized dystonia in *DYT1*-

positive patients was significantly higher than in *DYT1*-negative patients. The mean age at onset was 8.6 ± 1.6 years (7–12 years) in *DYT1*-positive patients, while mean age at onset in patients with no GAG deletion mutation was higher (15.7 ± 11.5 years). **Conclusions:** The *DYT1* 904–906 del GAG mutation is responsible for some of Iranian dystonia patients, and screening for the *DYT1* deletion is significant in cases with the generalized type of primary dystonia. Also, patients with leg or arm onset at a younger age are more likely to be *DYT1*-positive among primary torsion dystonia cases.

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Introduction

Dystonia is a group of movement disorders that are characterized by sustained muscle contractions causing twisting, repetitive movements or abnormal postures, affecting one or more sites of the body [1]. Dystonia is classified as either primary or secondary. Among primary dystonias, *DYT1* dystonia manifests autosomal dominant inheritance with low penetrance (30–40%) and is

Fig. 1. Comparison of a DYT1-positive patient's DNA with the normal sequence.

usually the only symptom of the disease [2]. It is caused by a mutation in the *DYT1* (also termed *TOR1A*) gene (9q34), which mainly leads to a 3-bp deletion (GAG) in exon 5 and subsequently results in loss of a glutamic acid residue in the protein torsin A [3, 4]. Primary dystonia is also termed primary torsion dystonia (PTD), and its onset typically is either during childhood (early onset around the age of 9 years) or in rare cases during adulthood (late onset at 45 years). Usually, disease onset is before the age of 26 years [1, 2]. In the secondary forms, dystonia is usually caused by an underlying disease which can be either genetic or environmental [5–7].

Dystonia can also be classified into different subtypes according to the part of the body affected: focal (affecting one body part, such as neck or leg), segmental (affecting two adjacent areas like head and neck), multifocal (two nonadjacent areas are involved, such as two arms), hemi-dystonia (when it is confined to one side of the body) and generalized [8, 9]. Most of PTDs begin with involvement of a limb in childhood with gradual progression to the generalized form [6].

The purpose of this study was to determine the rate of *DYT1* mutation in a consecutive series of Iranian patients with PTD, and to investigate the association of this mutation with phenotypic characteristics of dystonia, which are highly variable.

Subjects and Methods

Seventy-two unselected non-Jewish dystonia patients (42 males, 30 females) were examined by three neurologist physicians over 4 years; 9 were described as secondary dystonia patients due to birth incidents, limb injuries, an underlying disease or infection, or side effects of drugs [9, 10], and these patients were excluded from this study. The Ethics Committee of the Pasteur Institute of Iran approved this study. Following written informed consent for genetic studies from the remaining 63 patients, 10 ml peripheral blood was taken from each individual, collected in EDTA tubes and kept at -20°C . Whole-blood DNA extraction was carried out by standard method of salting out. Amplification of exon 5 of the *TOR1A* gene was performed by PCR using forward and reverse primers (*DYT1F* 5'-CCTGGAATACAAACACCTA-3', *DYT1R* 5'-GGCTGCCAATCATGACTGTC-3'). The PCR conditions for amplification of exon 5 of the *TOR1A* gene are as follows: one cycle at 94°C for 5 min, 94°C for 1 min, 60°C for 1 min, 72°C for 1 min in 32 cycles, and finally one cycle at 72°C for 5 min. Then sequencing was performed using ABI XL Sequencer (Applied Biosystems, Foster City, Calif., USA) as described elsewhere [11].

For statistical analysis, quantitative variables were expressed as means $\pm$ SD while qualitative variables were expressed as percentages.

Results

Ten cases out of 63 PTD patients (15.9%) were positive for GAG deletion in *DYT1* (*TOR1A*). The electrophoresis of a *DYT1*-positive patient in comparison with the normal

Table 1. Patients with positive DYT1 mutation

No./sex	AAE, years	AAO, years	Onset localization	Type of dystonia	Family history	Disease progression
1/M	16	12	R leg	generalized	negative	R leg, R hand, whole body
2/F	10	8	L fingers	generalized	negative	L fingers, L arm, waist, legs
3/M	9	NA ⁵	NA	NA	NA	NA
4/M	16	8	R leg	focal	negative	R leg
5/F	34	9	R leg	generalized	negative	R foot, R leg, waist, R hand, L foot
6/F	17	9	R hand	generalized	positive (siblings)	R hand, face, waist, R leg
7/F	13	NA	NA	NA	NA	NA
8/M	11	9	R toes	generalized	positive (2nd cousin)	R toes, R leg, trunk, R hand
9/M	8	7	waist	generalized	positive (2nd cousin)	waist, legs, neck
10/F	9	7	R leg	generalized	negative	R foot, R leg, trunk, L leg

AAE = Age at examination; AAO = age at onset; R = right; L = left; NA = not available.

Table 2. Site of onset and types of dystonia in PTD patients, compared with DYT1 mutation-negative and -positive status

	Available PTD patients		Negative patients		Positive patients	
	n (M:F)	%	n (M:F)	%	n (M:F)	%
Site of onset						
Foot, leg	12 (6:6)	25.00	7 (3:4)	17.5	5 (3:2)	62.5
Hand, arm	22 (12:10)	45.83	20 (12:8)	50.0	2 (0:2)	25.0
Neck	3 (1:2)	6.25	3 (1:2)	7.5	0	0
Cranial	5 (3:2)	10.42	5 (3:2)	12.5	0	0
Truncal	4 (3:1)	8.33	3 (2:1)	7.5	1 (1:0)	12.5
Multiple sites	2 (1:1)	4.17	2 (1:1)	5.0	0	0
Types of dystonia						
Focal	8 (5:3)	16.67	7 (4:3)	17.5	1 (1:0)	12.5
Segmental	7 (3:4)	14.58	7 (3:4)	17.5	0	0
Multifocal	6 (3:3)	12.50	6 (3:3)	15.0	0	0
Hemidystonia	3 (1:2)	6.25	3 (1:2)	7.5	0	0
Generalized	24 (14:10)	50.00	17 (11:6)	42.5	7 (3:4)	87.5
Total	48	100	40	100	8	100

M:F = Male:female ratio.

sequence is shown in figure 1. Clinical data were available for 48 (76.2%) of the 63 primary dystonia patients. In the DYT1-positive cohort, clinical data were available for 8 (80%) of the patients, and in the DYT1-negative cohort, clinical data were available for 40 (75.5%) (table 1).

The mean age of the DYT1-positive cohort, negative cohort and all available ($n = 48$) patients was 15.1 ± 8.4 (range 8–34), 22.3 ± 10.8 (range 6–54), and 21.1 ± 10.8 years (range 6–54), respectively. Almost all of these 48 patients were unrelated cases, except 2 (table 1), who were

second cousins. Among the positive patients, 3 had positive family history and all but 1 patient had the generalized type of dystonia; mean age at onset was 8.6 ± 1.6 years (table 1), while mean age at onset in DYT1 mutation-negative patients was higher (15.7 ± 11.5 years).

In all PTD patients, limbs were the most severely involved sites at the time of onset: hand and arm 45.83%; foot and leg 25%, and in most cases it had developed to the generalized form (50%). The difference between sites of onset in the majority of DYT1-positive cases in com-

Table 3. Types of dystonia in DYT1-positive and -negative patients with regard to their site of onset

Site of onset	Type of dystonia					Total
	focal	segmental	multifocal	hemidystonia	generalized	
Foot, leg	1 (P)	1 (N)	3 (N)	0	7 (3N, 4P)	12 (7N, 5P)
Hand, arm	4 (N)	3 (N)	3 (N)	1 (N)	11 (9N, 2P)	22 (20N, 2P)
Neck	2 (N)	1 (N)	0	0	0	3 (N)
Cranial	1 (N)	2 (N)	0	0	2 (N)	5 (N)
Truncal	0	0	0	0	4 (3N, 1P)	4 (3N, 1P)
Multiple sites	0	0	0	2 (N)	0	2 (N)
Total	8 (7N, 1P)	7 (N)	6 (N)	3 (N)	24 (17N, 7P)	48 (40N, 8P)

P = Positive patient(s); N = negative patient(s).

parison with negative patients was an outstanding result, which showed higher arm onset in *DYT1*-negative (50%) in contrast to higher leg onset in positive (62.5%) patients. Also, the progression to the generalized form of dystonia in *DYT1*-positive patients was significantly higher ($p = 0.047$) than in *DYT1*-negative patients (87.5% in *DYT1*-positives and 42.5% in *DYT1*-negatives). These data are summarized in table 2, and there are data about the latest type of dystonia – at the time of writing the article – in all available patients with regard to their sites of onset in table 3. In addition, in these 48 available patients, females had earlier onset of dystonia (12 ± 5.5) compared with males (15 ± 13), and except for those 3 positive patients none of them had a positive family history (table 1). We also had a rare finding: a late-onset negative patient with leg onset at the age of 48 whose disease had progressed to the generalized form within 6 years of onset (involving limbs, trunk, neck and face).

Discussion

Dystonia is a severe disease in most cases. Although primary dystonia is infrequent in the general population, it affects all aspects of a patient's life. Considering the fact that Jewish communities are closed with regard to marriage with non-Jewish populations, the likelihood of admixture with the Iranian population is very remote. To the best of our knowledge there has been no previous report about its clinical features, mutation screening, or genotype-phenotype correlation amongst people of Iran or neighboring countries prior to this report. We reported 63 Iranian non-Jewish patients with primary dystonia

with a high frequency of *DYT1* mutation, comparing with other populations [12–25].

In this study, mean age at onset of *DYT1* mutation-positive and *DYT1* mutation-negative patients was 8.6 ± 1.6 and 15.7 ± 11.5 , respectively. In comparison with some previous data, we had earlier age at onset [12–14, 26]. However, our patients showed a later age at onset than Italians [15]. The data about age at onset in females and males (females 12 ± 5.5 , males 15 ± 13) is also unlike most of previously published data, which showed earlier age at onset in males [16, 17, 27].

With regard to generalization rates (87.5% in positive and 50% in overall PTD patients), while most of our patients had generalized form, most patients in other studies have been reported with focal dystonia [17, 26, 28]. It might be because of our patients' earlier age at onset, since according to some data, later onset age may protect the patient from disease progression [18]. Alternatively, some patients with focal or even segmental dystonias may not have sought proper treatment and remained undiagnosed.

The patient with onset at 48 years was remarkable due to having generalized dystonia, which more often occurs in early-onset patients [12, 29], although there were also 3 patients in a German study who presented with generalized dystonia with age at onset over 40 years [28].

Overall, as a result of this study and according to the guidelines of the European Federation of Neurological Societies [18], molecular testing for GAG deletion in the *DYT1* gene is recommended in cases with early-onset primary dystonia regardless of family history, especially in those who suffer from the generalized type.

Conclusions

The *DYT1* 904–906 del GAG mutation was responsible for the dystonia of some Iranian patients, and screening for the *DYT1* deletion is important in cases with the generalized type of primary dystonia. Also, patients with leg or arm onset at a younger age were more likely to be *DYT1*-positive among primary torsion dystonia cases.

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References

- Fahn S, Marsden C, Calne D: Classification and investigation of dystonia. *Mov Disord* 1987;2:332.
- Bressman SB, de Leon D, Brin MF, Risch N, Burke RE, Greene PE, Shale H, Fahn S: Idiopathic dystonia among Ashkenazi Jews: evidence for autosomal dominant inheritance. *Ann Neurol* 1989;26:612–620.
- Goodchild RE, Dauer WT: The AAA+ protein torsinA interacts with a conserved domain present in LAP1 and a novel ER protein. *J Cell Biol* 2005;168:855–862.
- Ozelius LJ, Hewett JW, Page CE, Bressman SB, Kramer PL, Shalish C, de Leon D, Brin MF, Raymond D, Corey DP, Fahn S, Risch NJ, Buckler AJ, Gusella JF, Breakefield XO: The early-onset torsion dystonia gene (*DYT1*) encodes an ATP-binding protein. *Nat Genet* 1997;17:40–48.
- Albanese A, Barnes MP, Bhatia KP, Fernandez-Alvarez E, Filippini G, Gasser T, Krauss JK, Newton A, Rektor I, Savoia M, Valls-Solè J: A systematic review on the diagnosis and treatment of primary (idiopathic) dystonia and dystonia plus syndromes: report of an EFNS/MDS-ES Task Force. *Eur J Neurol* 2006;13:433–444.
- Fahn S, Bressman SB, Marsden CD: Classification of dystonia. *Adv Neurol* 1998;78:1–10.
- Valente EM, Warner TT, Jarman PR, Mathen D, Fletcher NA, Marsden CD, Bhatia KP, Wood NW: The role of *DYT1* in primary torsion dystonia in Europe. *Brain* 1998;121:2335–2339.
- de Carvalho Aguiar PM, Ozelius LJ: Classification and genetics of dystonia. *Lancet Neurol* 2002;1:316–325.
- http://www.wemove.org/dys/dys_anat.html.
- Schneider SA, Bhatia KP: Secondary dystonia – clinical clues and syndromic associations. *Eur J Neurol* 2010;17(suppl 1):52–57.
- Al Hashem G, Al Sweih N, Rotimi VO: Sequence analysis of *blacTX-M* genes carried by clinically significant *Escherichia coli* isolates in Kuwait hospitals. *Med Princ Pract* 2011;20:213–219.
- Im JH, Ahn TB, Kim KB, Ko SB, Jeon BS: *DYT1* mutation in Korean primary dystonia patients. *Parkinsonism Relat Disord* 2004;10:421–423.
- Lin YW, Chang HC, Chou YH, Chen RS, Hsu WC, Wu WS, Weng YH, Lu CS: *DYT1* mutation in a cohort of Taiwanese primary dystonias. *Parkinsonism Relat Disord* 2006;12:15–19.
- Major T, Svetel M, Romac S, Kostic VS: *DYT1* mutation in primary torsion dystonia in a Serbian population. *J Neurol* 2001;248:940–943.
- Zorzi G, Garavaglia B, Invernizzi F, Girotti F, Soliveri P, Zeviani M, Angelini L, Nardocci N: Frequency of *DYT1* mutation in early onset primary dystonia in Italian patients. *Mov Disord* 2002;17:407–408.
- Jamora R, Tan A, Tan L: A 9-year review of dystonia from a movement disorders clinic in Singapore. *Eur J Neurol* 2006;13:77–81.
- Naiya T, Biswas A, Neogi R, Datta S, Misra AK, Das SK, Ray K, Ray J: Clinical characterization and evaluation of *DYT1* gene in Indian primary dystonia patients. *Acta Neurol Scand* 2006;114:210–215.
- Harbo HF, Finsterer J, Baets J, Van Broeckhoven C, Di Donato S, Fontaine B, De Jonghe P, Lossos A, Lynch T, Mariotti C, Schöls L, Spinazzola A, Szolnoki Z, Tabrizi SJ, Tallaksen C, Zeviani M, Burgunder JM, Gasser T: EFNS guidelines on the molecular diagnosis of neurogenetic disorders: general issues, Huntington's disease, Parkinson's disease and dystonias. *Eur J Neurol* 2009;16:777–785.
- Dhaenens CM, Krystkowiak P, Douay X, Charpentier P, Bele S, Destée A, Sablonnière B: Clinical and genetic evaluation in a French population presenting with primary focal dystonia. *Mov Disord* 2005;20:822–825.
- Maniak S, Sieberer M, Hagenah J, Klein C, Vieregge P: Focal and segmental primary dystonia in north-western Germany – a clinico-genetic study. *Acta Neurol Scand* 2003;107:228–232.
- Kamm C, Naumann M, Mueller J, Mai N, Riedel L, Wissel J, Gasser T: The *DYT1* GAG deletion is infrequent in sporadic and familial writer's cramp. *Mov Disord* 2000;15:1238–1241.
- Kamm C, Castelon-Konkiewitz E, Naumann M, Heinen F, Brack M, Nebe A, Ceballos-Baumann A, Gasser T: GAG deletion in the *DYT1* gene in early limb-onset idiopathic torsion dystonia in Germany. *Mov Disord* 1999;14:681–683.
- Hjermind LE, Werdelin LM, Sorensen SA: Inherited and de novo mutations in sporadic cases of *DYT1*-dystonia. *Eur J Hum Genet* 2002;10:213–216.
- Matsumoto S, Nishimura M, Kaji R, Sakamoto T, Mezaki T, Shimazu H, Murase N, Shibasaki H: *DYT1* mutation in Japanese patients with primary torsion dystonia. *Neuroreport* 2001;12:793–795.
- Zhang SS, Fang DF, Hu XH, Burgunder JM, Chen XP, Zhang YW, Shang HF: Clinical feature and *DYT1* mutation screening in primary dystonia patients from south-west China. *Eur J Neurol* 2010;17:846–851.
- Lebre AS, Durr A, Jedynek P, Ponsot G, Vidailhet M, Agid Y, Brice A: *DYT1* mutation in French families with idiopathic torsion dystonia. *Brain* 1999;122:41–45.
- Sex-related influences on the frequency and age of onset of primary dystonia. Epidemiologic Study of Dystonia in Europe (ESDA) Collaborative Group. *Neurology* 1999;53:1871–1873.
- Grundmann K, Laubis-Herrmann U, Bauer I, Dressler D, Vollmer-Haase J, Bauer P, Stuhmann M, Schulte T, Schols L, Topka H, Riess O: Frequency and phenotypic variability of the gag deletion of the *DYT1* gene in an unselected group of patients with dystonia. *Arch Neurol* 2003;60:1266–1270.
- Szczaluba K, Jurek M, Milewski M, Friedman A, Kadziolka B, Szolna A, Bal J, Mazurczak T: Clinical characteristics of carriers of a GAG deletion in the *DYT1* gene amongst Polish patients with primary dystonia. *Eur J Neurol* 2007;14:659–662.