Neurogenetics (2001) 3:133–143
DOI 10.1007/s100480100111

ORIGINAL ARTICLE

**Joanne Chung-on Leung · Christine Klein
Jennifer Friedman · Peter Vieregge · Helfried Jacobs
Dana Doheny · Christoph Kamm · Deborah DeLeon
Peter P. Pramstaller · John B. Penney
Marvin Eisengart · Joseph Jankovic · Thomas Gasser
Susan B. Bressman · David P. Corey
Patricia Kramer · Mitchell F. Brin · Laurie J. Ozelius
Xandra O. Breakefield**

Novel mutation in the *TOR1A* (*DYT1*) gene in atypical, early onset dystonia and polymorphisms in dystonia and early onset parkinsonism

Received: 31 January 2001 / Accepted: 26 February 2001 / Published online: 19 May 2001

Abstract Dystonia is a movement disorder involving sustained muscle contractions and abnormal posturing with a strong hereditary predisposition and without a distinct neuropathology. In this study the *TOR1A* (*DYT1*) gene was screened for mutations in cases of early onset dystonia and early onset parkinsonism (EOP), which frequently presents with dystonic symptoms. In a screen of 40 patients, we identified three variations, none of which occurred in EOP patients. Two infrequent intronic single base pair (bp) changes of unknown consequences were found in a dystonia patient and the mother of an EOP patient. An 18-bp deletion (Phe323_Tyr328del) in the *TOR1A* gene was found in a patient with early onset dystonia and myoclonic features. This deletion would remove 6 amino acids close to the carboxy terminus, including a putative phosphorylation site of torsinA. This 18-bp deletion is the first additional mutation, beyond the GAG-deletion (Glu302/303del), to be found in the *TOR1A* gene, and is associated with a distinct type of early onset dystonia.

Keywords Early onset parkinsonism · Myoclonus-dystonia · *TOR1A* (*DYT1*) · Multiphor single-strand conformational polymorphism analysis · 18-bp deletion

J.C. Leung · C. Klein · J. Friedman · C. Kamm · J.B. Penney
X.O. Breakefield (✉)
Department of Neurology,
Massachusetts General Hospital and Harvard Medical School,
Boston, MA 02114, USA
e-mail: breakefi@helix.mgh.harvard.edu
Tel.: +1-617-7265728, Fax: +1-617-7241537

C. Klein · P. Vieregge · H. Jacobs
Department of Neurology, Medical University of Lübeck,
Lübeck, Germany

D. Doheny · M.F. Brin
Movement Disorders Center, Mount Sinai Hospital,
New York, NY 10029, USA

D. DeLeon · S.B. Bressman
Department of Neurology, Beth Israel Medical Center,
New York, NY 10003, USA

P.P. Pramstaller
Department of Neurology, Regional General Hospital,
Bolzano-Bozen, Italy

J. Jankovic
Department of Neurology, Baylor College of Medicine,
Houston, TX 77030, USA

T. Gasser
Department of Neurology,
Klinikum Grosshadern, Ludwig-Maximilians-University,
Munich, Germany

D.P. Corey
Howard Hughes Medical Institute,
Massachusetts General Hospital and Neurobiology Department,
Harvard Medical School, Boston, MA 02114, USA

P. Kramer
Department of Neurology,
Oregon Health Sciences University, Portland,
OR 97201, USA

M. Eisengart
MaineGeneral Medical Center, Waterville, ME 04901, USA

L.J. Ozelius
Department of Molecular Genetics,
Albert Einstein College of Medicine, Bronx, NY 10461, USA

X.O. Breakefield
Massachusetts General Hospital-East,
Department of Molecular Neurogenetics,
13th Street, Building 149, 6th Floor, Charlestown,
MA 02129, USA

Introduction

Dystonia is a state of sustained muscle contractions and abnormal posturing. It is seen in neurological conditions in which dystonia is the primary feature (primary dystonia) and in a number of neurodegenerative diseases, such as Parkinson's disease, in which it is one of a set of symptoms [1]. At least 12 different gene loci have been implicated in the primary dystonias [2, 3]. Four non-degenerative types and one degenerative type of hereditary dystonic syndromes can manifest parkinsonian features. These include rapid-onset dystonia parkinsonism [4] that maps to 19q [5], an autosomal dominant form of dopa-responsive dystonia (DRD) [6] that maps to 14q [7], caused by mutations in the gene for GTP cyclohydrolase I [8], an autosomal recessive form of DRD that maps to 11p [9], caused by mutations in the gene for tyrosine hydroxylase [10, 11], and X-linked dystonia-parkinsonism [12] that maps to Xq [13]. Parkinson's disease is a neurodegenerative condition with early onset (juvenile), and even some late-onset, cases being caused by mutations in the gene for parkin, a ubiquitin-protein ligase [14, 15, 16].

In patients with onset of parkinsonism prior to 50 years of age (EOP), the first manifestations are frequently dystonic symptoms, with subsequent progression with age to classic features of Parkinson's disease, including tremor, rigidity, and bradykinesia [17]. These observations led us to hypothesize that the young brain (<30 years of age) may be especially sensitive to low levels of dopamine release into the striatum, resulting in dystonic symptoms, with continuing low levels throughout life sustaining a dystonic phenotype. In contrast, the striatum appears less sensitive to low levels of dopamine commencing in adulthood, but further decreases caused, for example, by extensive loss of dopaminergic neurons, produce symptoms of parkinsonism. The theoretical link between dystonia and parkinsonism is strengthened by the finding that the highest levels of *TOR1A* messenger RNA are found in the dopaminergic neurons of the substantia nigra that innervate the striatum [18, 19] and die in Parkinson's disease. Furthermore, immunostaining for torsinA in autopsy of brains from patients with Parkinson's disease [20] and diffuse Lewy body disease [21] show association of torsinA with α-synuclein in Lewy bodies.

Most cases of early onset generalized dystonia (about 70%) are caused by the same three base pair (bp) (GAG) deletion in the *TOR1A* (*DYT1*) gene encoding torsinA [22, 23]. The GAG deletion is found predominantly in patients with typical early onset dystonia [24]. Thus, loss of a specific glutamic acid residue in the carboxy terminus of torsinA, when present in the heterozygous state, appears to cause a unique change in protein function, resulting in a fairly predictable phenotype, albeit with a reduced penetrance of 30%–40% [25]. Based on the theory that dystonia and parkinsonism share the common feature of reduced dopamine neurotransmission in the striatum, we explored the possibility that other mutations in this gene might have a role in cases of EOP that present with dystonia. A previous mutational screen of *TOR1A* did not reveal any additional changes in the coding region or splice junctions in 17 cases of typical early onset, generalized dystonia who did not have the GAG deletion, 5 dystonic individuals with homozygosity for multiple polymorphic markers in the region of chromosome 9q34 bearing this gene, or a representative Ashkenazic Jewish individual with late-onset dystonia who shared a common haplotype in this chromosomal region with other late-onset individuals in this ethnic group [26]. These results suggested a founder mutation [26]. Furthermore, no additional changes have been observed in the coding region of this gene during assessment of the GAG deletion in over 320 Ashkenazic Jewish and 137 non-Jewish control samples, as well as over 2,000 individuals with various forms of dystonia, including 57 from Germany [27], 100 from France [28], 150 from the United Kingdom [29], and 1,800 from the United States (unpublished data). The possibility remains that other mutations in *DYT1* causing dysfunction of torsinA could interfere with function or survival of dopaminergic neurons.

In this study, mutational analysis of the *TOR1A* gene was carried out in 15 individuals with early onset dystonia, some with other neurological features including myoclonus (rapid jerking movements), chorea, ataxia, and parkinsonism, and in 24 individuals with EOP, which typically begins with dystonic features and progresses to a classic parkinsonian phenotype. Analysis was carried out by PCR amplification of exonic and flanking intronic sequences followed by single-strand conformational polymorphism (SSCP) analysis [30] using the Multiphor II Electrophoresis Unit (Pharmacia Biotech) [31] and DNA sequencing of shifted bands. In addition, RT-PCR of mRNA and nested PCR analysis of resulting cDNA were carried out to evaluate message processing in the 1 dystonic sample showing a single bp change at an intronic region.

Materials and methods

Patient ascertainment and clinical evaluation

Subjects (Table 1) were acquired through Neurology Services at Lübeck University Hospital, Lübeck, Germany; Ludwig Maximilians University, Munich, Germany; Medical Center, General Regional Hospital, Bolzano, Italy; Massachusetts General Hospital, Boston, Massachusetts; MaineGeneral Medical Center, Waterville, Maine; Beth Israel Medical Center, New York; Columbia University Medical Center, New York; Mount Sinai Medical Center, New York; and Baylor College of Medicine, Houston, Texas. After obtaining informed consent for participation in genetic research all patients underwent a standardized neurological history, examination, and routine laboratory testing.

Representative samples were chosen from patients with a variety of movement disorders. Patients were classified as EOP if they had onset of parkinsonian signs/symptoms (dystonic movements, bradykinesia, rigidity, tremor, and/or postural instability) beginning below the age of 50 years. The 24 EOP patients screened in this study were selected from a population of 127, as a random subset with dystonia as the initial symptom and the primary neurological feature over several years of observation. All 15 atypical

dystonia patients, except 1, had onset before 28 years. Other neurological features seen in these patients included myoclonus, tremor, chorea, mental retardation, seizures, and bradykinesia (Table 1).

DNA was extracted from venous blood samples or lymphoblasts by standard procedures (Nucleon II DNA extraction Protocol, Tepnel Life Sciences, Manchester, UK).

Amplification of short PCR fragments for SSCP

PCR reactions were performed on genomic DNA samples of all 40 individuals (Table 1) using nine primer sets (Table 2) designed to produce fragments not larger than 300 bp that covered the whole *DYT1* coding sequence in overlapping fragments. Amplification conditions are listed in Table 2. PCR products were first resolved by electrophoresis in 1.0% agarose gels (IBI Agarose, International Biotechnologies) to ensure successful amplification, and then diluted with denaturing solution to about 100 ng DNA in 10 µl total volume with loading dye for multiphor SSCP analysis (Pharmacia Biotech Instructions Manual for the ExcelGel DNA Analysis Kit). Following denaturation (95°C, 5 min), samples were loaded onto the ExcelGel (Pharmacia Biotech), pre-cooled to 4°C by the Multiphor II Electrophoresis Unit (Pharmacia Biotech) with the horizontal electrophoresis chamber connected to a cooling module (MGW Lauda RM20). Electrophoresis was carried out at 600 V, 15 W, and 50 mA. The ExcelGel was stained using a modified silver staining protocol [32].

Analysis of the SSCP gels and sequencing

Banding patterns of denatured DNA PCR fragments were compared across samples (including non-denatured control) to detect shifted bands or altered patterns that might indicate potential sequence changes. In cases of DNA fragments bearing previously described single bp polymorphisms, i.e., those generated with primer sets H41/H47 (exon 1), H38/6548 (exon 2), and H14/7103 (exon 4) [22] (Fig. 2), at least two representatives of each polymorphic pattern were sequenced to determine whether their SSCP pattern corresponded to the known polymorphisms. PCR products containing other novel changes were resolved on agarose gels and extracted either by QIAquick Gel Extraction Kit (Qiagen, Calif., USA) or a silane-treated glass wool spinning column (Alltech Associates, Ill., USA) before sequencing with the Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit (USB, Ohio, USA). Sequencing reactions were resolved on 6% monomer SequaGel-6 (National Diagnostics, Ga., USA) followed by autoradiography. Sequences were read manually and compared with the genomic DYT1 sequence (Accession no. AL158207). Ambiguities or possible changes were all sequenced again in the opposite direction.

Detection of possible splice variants was performed on the total RNA extracted from lymphoblast line, GUS 27834, which showed a single bp change in intron 2, using Gibco's M-MLV reverse transcriptase (Life Technologies) with random hexamers and primer 6418 (Table 2) to amplify the cDNA of the *DYT1* gene. Full-length *DYT1* coding sequence was then PCR amplified with primers H50 (ATGAAGCTGGGCCGGGCCGTG) and H51 (TCAATCATCGTAGTAATAATCTAA). Nine internal PCR reactions that amplified across the exon 2-exon 3 junction on the cDNA were prepared for detection of potential splice variants. Primer sets H50/7051, H50/6550, H50/H42, 6343/7051, 6343/6550, 6343/H42, 6441/7051, 6441/6550, and 6441/H42 were used respectively [H50 (ATGAAGCTGGGCCGGGCCGTG), 6441 (GGCTACATCTACCCGCGT), 6343 (TCTCACGCTCTCCCTGCACGG); 7051 (TGAAGATGGACCTCGCACAG), 6550 (TGAGAAATATGAACATGGC), and H42 (GAGGTTCCGGTCAATTAAGC)]. PCR products were then run on 1.5% agarose gel for detection of altered fragment sizes, indicative of splice variations.

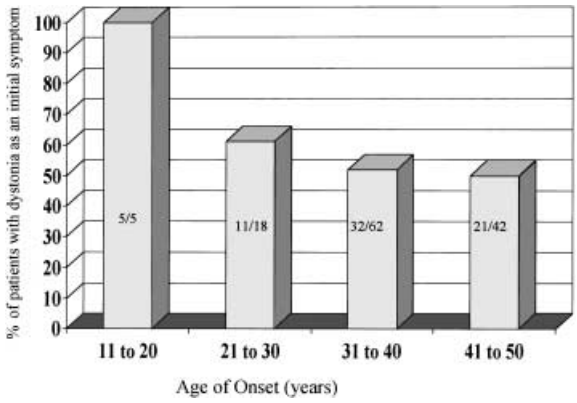


Fig. 1 Early onset Parkinson patients with dystonia as the presenting symptom as a function of age of onset

Results

Clinical features of patients

Patients were included in three categories: (1) those with classic features of Parkinson's disease who had presented with dystonic posturing prior to the age of 50 years, and who were negative for mutations in the gene for parkin by SSCP screening [two patients, L-359 and L-364, were subsequently found to have mutations in the gene for parkin (C. Klein et al., in preparation)]; (2) those with early onset (<28 years) dystonia with and without atypical features who were negative for the GAG deletion, 1 of whom (GUS 26733) had parkinsonian symptoms; and (3) a single patient (GUS 27678) with late-onset dystonia (39 years) with atypical features, also negative for the GAG deletion, who had two siblings with dystonia, one with early onset. (Most dystonia patients were not screened for parkin mutations.)

Dystonic symptoms in Parkinson patients as a function of age of onset

The 24 EOP patients whose DNAs were evaluated in this study came from a population of 127 patients with classic Parkinson's disease with onset before the age of 50 years [33]. In this larger population a direct correlation was noted between the age of the patient at the time of presentation with neurological symptoms and tendency for the presenting symptom to be dystonia (Fig. 1).

Detection of sequence changes

SSCP analysis was used to screen for mutations in *TOR1A* using genomic DNA. This method resolves single-stranded DNA fragments by their configuration in non-denaturing gels. All five exons of *TOR1A* were screened by SSCP analysis in the 40 patients (Table 1) using the primer sets listed in Table 2, except for the 3'

Table 1 Clinical information of all the patients participating in this study (*AR* alcohol responsive, *B* bradykinesia, *CH* chorea, *CR* cogwheel rigidity, *D* depression, *DR* dopa responsive, *H* hyperkinesia, *M* myoclonus, *MF* motor fluctuations, *MR* mental retardation, *PRI* postural reflex impairment, *T* tremor, *RT* resting tremor, *S* seizures, *NA* not applicable)

	Patient	Age of onset	Site of onset	Clinical syndrome	Dystonic symptom	FH[d]	Ethnicity	Sex	Associated neurological features
EOP	L-359[a]	15	Left	EOP	+	+	German	M	B, RT, CR
	L-333	16	Right hand	EOP	+	–	German	M	B, RT, CR, PRI, H, MF
	L-310	23	Right hand	EOP	–	–	German	M	B, RT, CR, PRI, H, MF
	L-363	24	Right hand	EOP	+	–	German	M	B, RT, PRI, H, MF
	L-361	24	Right hand	EOP	+	–	German	M	BR, CR
	L-351	25	Right hand	EOP	–	–	German	M	BR, RT, CR, PRI, severe H, MF
	L-345	30	Right hand	EOP	+	+	German	F	B, RT, CR
	L-353	31	Left hand	EOP	+	+	German	F	B, CR, RT, MF, D, H
	L-365	31	Left hand	EOP	–	+	German	F	B, CR, PRI, H, D
	L-341	31	Right hand	EOP	+	+	German	F	WC, B, RT, CR, PRI
	L-342	32	Left hand	EOP	–	–	German/Czech	M	B, RT, CR, PRI, possible lyme-encephalitis
	L-364[b]	33	Left hand	EOP	+	+	German	F	B, RT, CR
	L-370	34	Right arm	EOP	+	+	German	F	B, CR, H
	L-401	34	Right leg	EOP	–	–	German	M	B, CR
	B-106	34	Right	EOP	–	+	Italian/South Tyrol	M	B, CR
	L-356	35	Left hand	EOP	+	–	German	M	B, RT, CR, PRI, H
	GUS 27466	35	Upper limbs	EOP	+	+	Puerto Rican	M	B, RT, PRI, D, MF, H
	L-374	36	Left arm	EOP	–	+	German	M	B, RT, CR, PRI, H, MF
	L-252	37	Right limbs	EOP	+	+	German	M	B, CR
	L-299	37	Right hand	EOP	+	–	German	F	B, CR, PRI, D
	L-274	38	Right hand	EOP	–	+	German	F	B, CR, RT, D
	L-358	42	Right arm	EOP	+	+	German	M	B, RT, CR, PRI
	GUS 27730	44	Left arm + head/neck	EOP	+	–	Hispanic	M	NA
	L-383	45	Left leg	EOP	+	+	German	M	B, RT, CR, restless legs syndrome, rigidity
	GUS 28022[c]	NA	NA	Asymptomatic	–	+	Puerto Rican	F	NA

Table 1 (continued)

	Patient	Age of onset	Site of onset	Clinical syndrome	Dystonic symptom	FH[d]	Ethnicity	Sex	Associated neurological features
Dystonia	GUS 17859	1	Feet	Generalized dystonia	+	+	Polish/Italian	M	DR
	GUS 28206	3	Right arm	Generalized/myoclonus dystonia	+	+	Italian	F	M
	MIN 6830	3	Leg	Generalized dystonia	+	–	Chinese-Puerto Rican	F	Ataxia, dysarthria, left leg weakness, jerky movement disorder, poorly characterized
	99756	8	Larynx	Generalized dystonia	+	+	German	M	NA
	GUS 27834	9	Legs	Generalized dystonia	+	–	Puerto Rican	M	CH, T, jerky movement disorder, poorly characterized
	GUS 15166	17	Right elbow	Generalized dystonia	+	+	Cherokee-Indian	F	DR
	GUS 28086	27	Right arm	Generalized dystonia	+	unknown	Caucasian[e]	F	M, T
	GUS 5097	Chil-hood	Hand	Multifocal dystonia	+	+	Ashkenazi Jewish	M	Scoliosis
	GUS 27847	9	Arms, speech	Multifocal dystonia	+	–	Jewish	F	NA
	GUS 27678	39	Right arms	Multifocal dystonia	+	+	French-Canadian	F	S, MR, T
	GUS 26733	6	Right hand	Mixed movement disorder	+	+	Greek	F	M, RT, CH, MR, S, parkinsonian features
	MIN 7088	2	Right arm	Myoclonus dystonia	+	+	Irish/German	M	M
	MIN 5024	5	Legs	Myoclonus dystonia	+	+	Polish/ Lithuanian/Italian	F	M
	MIN 5256	5	Arms	Myoclonus dystonia	+	+	Caucasian[e]	M	AR, T, M
	MIN 4891	8	Hand	Myoclonus dystonia	+	+	Scottish	M	M, AR

[a] Patient L-359 has a mutation in exon 7 of the gene for parkin (Klein et al., in preparation)
[b] L-364 has a mutation in exon 2 of the gene for parkin (Klein et al., in preparation)
[c] GUS 28022 is the mother of an EOP patient with another mutation in exon 2 of the gene for parkin (Klein et al., in preparation)
[d] Family history of movement disorder
[e] Ethnicity was not recorded for these patients

Table 2 Primers used to amplify short fragments of *TOR1A* gene (*DMSO* dimethyl sulfoxide)

Exon	Location of primer	Name of primer	Primer sequence (5' to 3')	PCR conditions	Product size (bp)
1	5' UTR	H41	gcaaaacagggctttgtaccg[a]	94°C (2 min); [94°C (1 min); 55°C (1 min); 72°C (1 min 30 s)] 35 cycles; 72°C (10 min) with 10% DMSO	298
	Exon 1	H47	agtagagacgcgggtagatg[a]		
	Exon 1	7050	gcgtctctactgcctcttcg[a]	94°C (2 min); [94°C (1 min; 58°C (1 min); 72°C (1 min 30 s)] 40 cycles; 72°C (10 min)	138
	Intron 1	H18	atgccctggtcctagttcag[a]		
2	Intron 1	H38	ggtttcgcaaggtgcttgga[a]	94°C (2 min); [94°C (1 min); 55°C (1 min); 72°C (1 min 30 s] 35 cycles; 72°C (10 min)	247
	Exon 2	6548	ccgtgcagggagagcgtgag		
	Exon 2	6343	tctcacgctctccctgcacgg	94°C (2 min); [94°C (1 min); 55°C (1 min); 72°C (1 min 30 s] 35 cycles; 72°C (10 min)	184
	Intron 2	H24	gggattccaaacttccatcc		
3	Intron 2	H16	tagaaggagctgattgatggc	94°C (2 min); [94°C (1 min); 55°C (1 min); 72°C (1 min 30 s] 40 cycles; 72°C (10 min)	300
	Intron 3	H15	tcctggagctcagaggcttg		
4	Intron 3	H14	agcctctgagctccaggag	94°C (2 min); [94°C (1 min); 60°C (1 min); 72°C (1 min 30 s] 35 cycles; 72°C (10 min)	270
	Intron 4	7103	gatgctgacagtgaccctg		
5	Intron 4	H9	gacccccagtagacgtttgt[a]	94°C (2 min); [94°C (1 min); 55°C (1 min);) 72°C (1 min 30 s] 35 cycles; 72°C (10 min	230
	Exon 5	6203	cgggactgcatttccsctc		
	Exon 5	6419	cctggaatacaaacaccta	94°C (2 min); [94°C (1 min); 55°C (1 min); 72°C (1 min 30 s] 35 cycles; 72°C (10 min)	254
	3'UTR	6418	ggtggaaggactgagtgttg		
	Exon 5	6419	same as above	94°C (2 min); [94°C (1 min); 55°C (1 min); 72°C (1 min 30 s] 40 cycles; 72°C (10 min)	225
	3'UTR	H48	ggctgccaatcatgactgtc		

[a] From Ozelius et al. [26]

end of exon 1 (primers 7050/H18). That region is very GC rich and contains repetitive sequences [26], which proved difficult to amplify under a number of conditions using several sets of primer pairs, and was therefore not thoroughly analyzed in the current screen.

Sequence variations in sample set

In addition to one major deletion, two subtle shifts on the SSCP gels gave indications of previously unknown sequence changes (Fig. 2). First, a single bp change in the 5' UTR in a heterozygous state (-IIIC>T) was observed in the mother (GUS 28022) of an EOP patient. This patient did not have this polymorphism, but was later found to have a mutation in the gene for parkin, *PARK2* [34]. This polymorphism in *TOR1A* was not observed in 50 controls or other samples tested here (termed, extended sample set). No alteration in splicing is predicted based on the simple AG-GT consensus sequences flanking introns [35]. Secondly, a single bp change in intron 2 in a heterozygous state (445-22G>A) in 1 dystonia patient (GUS 27834) with atypical features, including tremor and chorea, and not in the extended sample set. This change did not affect the canonical splice junction sequences, and RT-PCR did not reveal any apparent splice alterations in the message, although a mis-spliced message might be unstable and thus undetectable by this method.

Mutation in myoclonus-dystonia patient

One patient with early onset dystonia and myoclonic features (MIN 5024) showed a marked change in the SSCP banding pattern for exon 5 (Fig. 3a). Sequencing revealed an 18-bp deletion (Phe323_Tyr328del) close to the stop codon in a heterozygous state, confirmed by sequencing of both strands (Fig. 3c). The deleted sequence GTTCACCAAGTTAGATTA (966_983del) predicts loss of the amino acid sequence, FTKLDY, in the carboxy terminus of torsinA (Fig. 4). This deleted allele can also be resolved from the normal allele as a distinct band by electrophoresis in 1.5% agarose gels (Fig. 3b). This deletion, which is revealed by a marked band shift in genotyping using the set of PCR primers routinely used for screening for GAG deletions [23], has not been seen in over 1,800 DNA samples from dystonic individuals analyzed in our laboratory nor in more than 482 controls. Six people from the family of MIN 5024 underwent standardized neurological examination and DNA analysis. In addition to MIN 5024, her brother, mother, and maternal grandfather also carry this 18-bp deletion in a heterozygous state (Fig. 5). These other carriers showed

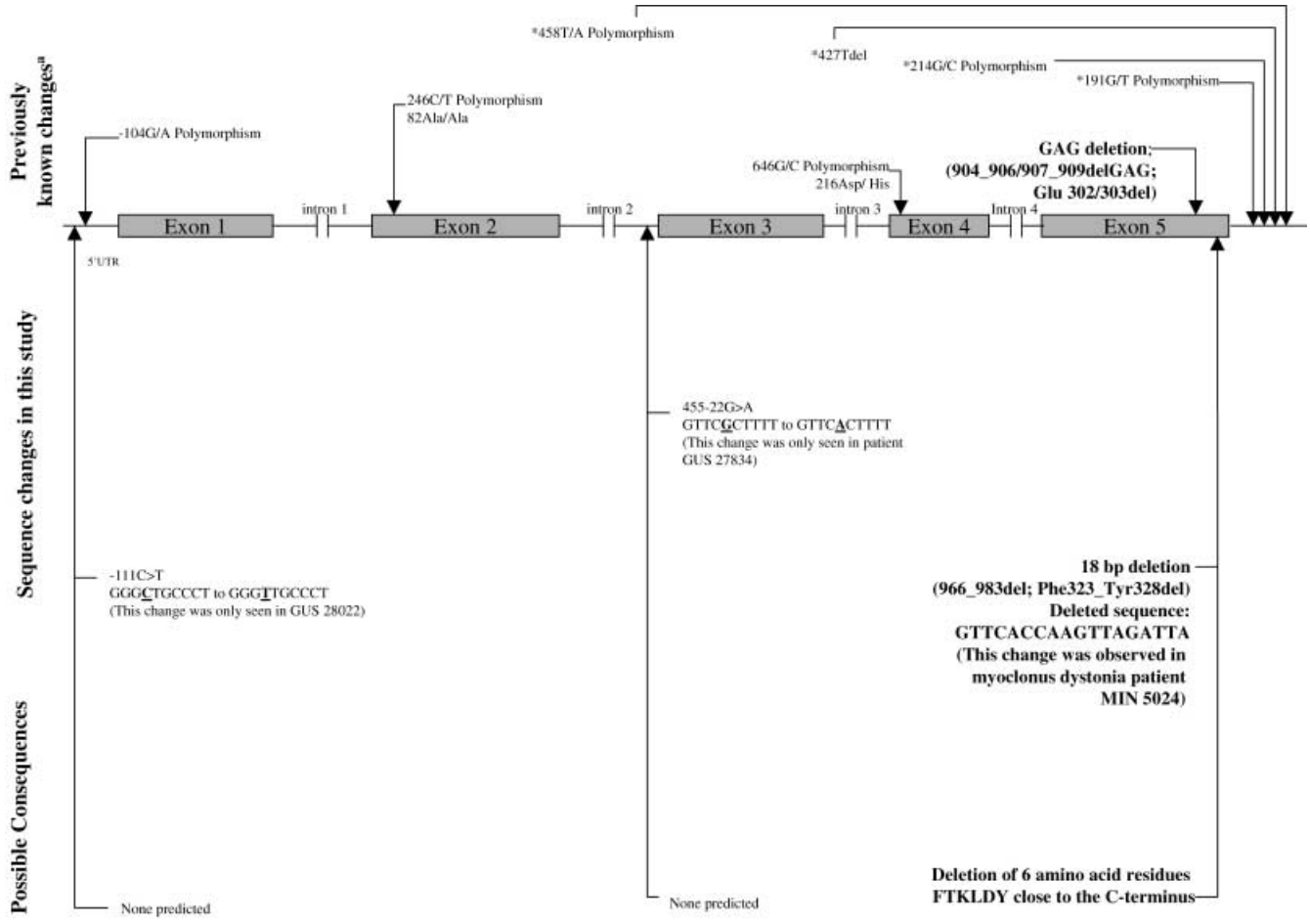


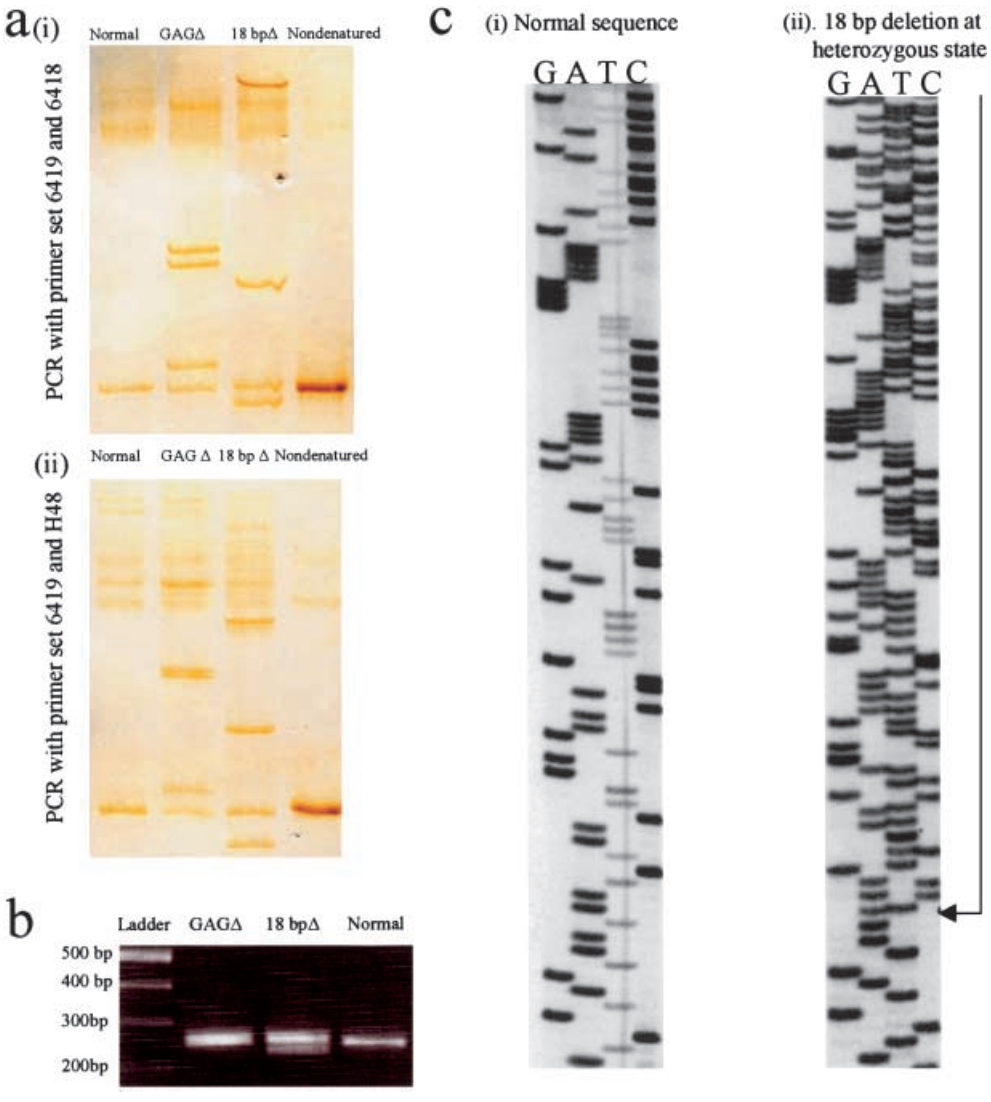
Fig. 2 Sequence variations in *TOR1A* gene. Map of *TOR1A* gene showing positions of sequence changes (from top to bottom): previously reported sequence changes [22, 26], sequence changes found in this study, and predicted consequences. The allele frequency of the 646G/C (216Asp/His) polymorphism in exon 4 in the population of 24 EOP patients is 93.75% "G" and 6.25% "C"; in the 15 dystonia patients the frequencies are 86.6% "G" and 13,3% "C"; while in both control non-Jewish and Ashkenazi Jewish populations of 30 people each the frequencies are 88.0% "G" and 12.0% "C"

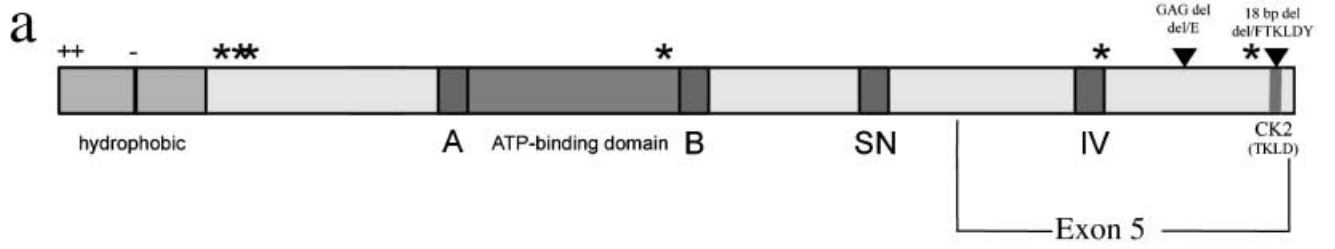
possible neurological features related to dystonia and myoclonus, but the father of MIN 5024 lacked the deletion and also had possible myoclonus (D. Doheny and M. Brin, personal communication). These findings are consistent with autosomal dominant transmission of early onset, atypical dystonia in this family with reduced penetrance of the 18-bp deletion.

Discussion

TorsinA, a novel member of the AAA+ chaperone family, underlies most cases of dominantly inherited, early onset generalized dystonia [22, 36]. Remarkably, in a screen of over 1,800 patients with dystonia, prior to the present study, only one mutation has been identified, a GAG deletion (Glu302/303del), which would result in loss of a single glutamic acid in the carboxy terminus [22, 23, 24, 26]. The present study describes another mutation in *TOR1A* in a patient with atypical early onset dystonia that is predicted to delete six amino acids in the carboxy terminus, 21 amino acids downstream from the glutamic acid deletion. This new mutation would remove a predicted casein kinase 2 phosphorylation domain in the protein [22] (Fig. 4) and is quite rare, being found in only a single patient with dystonia and not in controls or patients with EOP. Of the collective 11 sequence variations noted to date in *TOR1A* ([26]; this study), 7 appear to be normal polymorphisms, as they were also found in control samples. Three variations, including the 2 deletions (GAG and 18-bp), and 1 bp change in an intron, were seen in dystonia patients and not in control samples. The single bp change may represent a rare polymorphism or a mutation that affects transcription, processing, or stability of the torsinA RNA.

TorsinA is a member of a novel subfamily of AAA+ proteins conserved throughout many species with unknown function [26]. Its membership of the AAA+ chaperone family suggests a role in manipulating the configuration of other proteins, for example in recovery from cellular stress or movement/fusion of vesicles [37]. The two deletion mutations, which have been observed in this protein to date, both produce the neurological symptoms of early onset, primary dystonia in the heterozy-





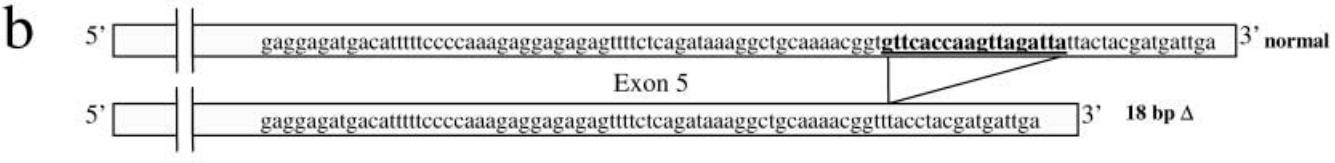


Fig. 3 Deletion mutations in *TOR1A*. **a** Multiphor single-strand conformational polymorphism analysis visualized by silver staining showing band patterns of PCR products of a normal, GAG-deleted patient, the 18-bp-deleted patient, and a non-denatured sample in the 3' end of exon 5 of *TOR1A* [with primer sets 6419/6418 (*i*) and 6419/H48 (*ii*), respectively]. **b** PCR products of the 3' end of exon 5 (with primer sets 6419/6418) visualized by ethidium bromide on 1.5% agarose gel. The 18-bp-deleted allele can be resolved from the normal allele. **c** Sequence (*i*) of a normal individual and (*ii*) MIN 5024 with the 18-bp deletion using primer 6418. The *arrow* shows where the "double sequence" starts, indicating one normal and one deleted allele

c Carboxy terminal 40 amino acids sequence of TorsinA

```
                                  Normal :   DEDIVSRVAEEMTFFPKEERVFSDKGCKTVFTKLDYYYDD
      GAG deletion (Glu302/303del) :   DEDIVSRVA*EMTFFPKEERVFSDKGCKTVFTKLDYYYDD
18 bp deletion (Phe323_Tyr328del) :   DEDIVSRVAEEMTFFPKEERVFSDKGCKTV******YYDD
```

gous state through dominant transmission with reduced penetrance (D. Doheny and M. Brin, personal communication; [25]). These mutations should aid in establishing structure/function relationships in this protein. Although it remains possible that other mutations in the heterozygous or homozygous state may cause a different disease state, such as Parkinson's disease, no evidence for this was found here in a screen of 24 EOP patients. A 24-bp repeat domain near the GAG deletion may account for its apparently high mutability [38]; there is no repeated element near the 18-bp deletion, and it appears to be much rarer. It is remarkable that these mutations both consist of in-frame deletions in the carboxy terminus of the protein. Comparison of conserved amino acid sequences across members of the AAA+ family show that the glutamic acid deletion falls within a conserved domain [22], defined as the box VIII sensor 2 domain by Neuwald et al. [36], based on an overlay of the predicted secondary structure with that of crystallized *N*-ethylmaleimide sensitive factor, another member of the AAA+ family. This domain is predicted to have an alpha helix configuration in torsinA and may participate in hexamer formation [36]. The deletion of a single or multiple amino acids in the carboxy terminus of torsinA would change the relative position and charge of the alpha helix in this region, which could affect hexamer formation or binding to interacting proteins. The removal of a putative threonine phosphorylation site, TKLD, by the 18-bp deletion (Fig. 4) could also affect regulated functions of the protein. The question remains as to whether these mutations disrupt the action of normal torsinA in a dominant-negative manner, or confer a novel function to the mutant protein (gain-of-function).

Recent studies on overexpression of GAG-deleted torsinA in cultured cells reveal the formation of cytoplasmic inclusion bodies, that are not seen with overexpression of the normal protein [37, 39]. These inclusions represent membrane whorls apparently derived from the endoplasmic reticulum (ER), and may interfere with membrane trafficking in cells by their physical bulk and/or disrupt ER functions or vesicle movement [39]. It is not known whether such inclusions form in the brains of patients with early onset of dystonia, but neuropathological studies to date indicate no obvious degeneration of neurons [40, 41].

The phenotype of the GAG deletion in torsinA in the heterozygous case (no homozygous cases have been reported) is quite distinct with onset in childhood (mean age 12 years) usually starting in the foot or arm and progressing to involve other limbs and the torso within a 5-year period [24]. Rarely does onset occur after 28 years of age, suggesting a window of susceptibility during postnatal development. Although the majority of patients carrying the GAG-deleted *TOR1A* gene manifest early onset generalized dystonia, less commonly focal dystonia such as writer's cramp, torticollis and spasmodic dysphonia [23, 24, 42, 43] may be associated features, or patients may display only focal dystonia, including torticollis and spasmodic dyphonia [23, 24, 42, 43]. This paper describes the first case of neurological dysfunction caused by a mutation in *TOR1A* other than the GAG deletion. In this patient, onset of dystonia began early (8 years) in the limbs with subsequent generalization and features of myoclonus and tics (D. Doheny, M. Brin, personal communication). Of the various inherited forms of dystonia that have been described [2, 3], one, DYT11, termed myoclonus-dystonia, is inherited in an autosomal dominant manner with reduced penetrance and onset usually in adolescence or young adulthood [44, 45]. Two genes other than *TOR1A* have been implicated in families with this form of dystonia – an as yet unidentified locus on chromosome 7 [46, 47], which appears to be the major locus, and the D2 dopamine receptor gene on chromosome 11 with an associated missense change in one affected family [48]. The occurrence of myoclonic symptoms in patients with early onset, generalized dystonia, as observed in the present study, is relatively rare, but supports a molecular/cellular continuum underlying the various forms of dystonia.

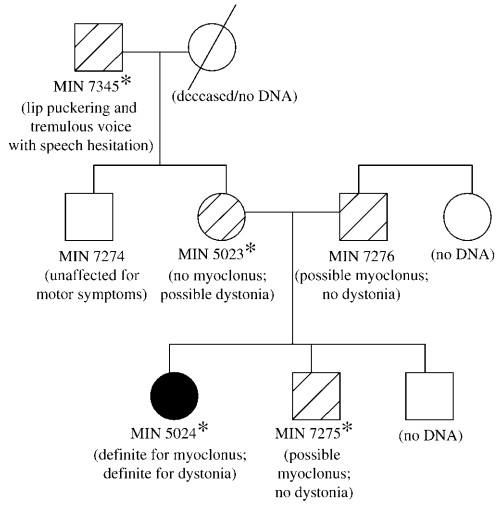


Fig. 5 Family of the myoclonus-dystonia patient, MIN5024. Individuals with the 18-bp deletion in the heterozygous state are represented by an *asterisk*. *Shaded* or *diagonally striped* symbols indicate phenotype: shaded=definitely affected, striped=possibly affected. (Only the individuals assigned a MIN number were neurologically examined and screened for the GAG and 18-bp deletion)

◀ **Fig. 4** Predicted changes in torsinA. **a** Schematic representation of torsinA domains. The *N*-terminal region (*left*) contains about 40 hydrophobic amino acids, preceded by 2 basic residues (*K*, *R*) and bisected by a polar and an acidic residue (*Q*, *E*). The ATP-binding domain is indicated along with its conserved A and B motifs. Two additional motifs conserved with the HSP100 family (*SN* and *IV*) are shaded. Conserved cysteine residues are represented by an *asterisk*. The *solid triangles* represent the site of the GAG (Glu302/303del) deletion and the 18-bp GTTCACCAAGTTAGATTA (Phe323_Tyr328del; deletion of FTKLDY) deletion in torsinA (adapted from Ozelius et al. [49]). **b** Drawing showing the normal and heterozygous state of the 18-bp deletion sequences in exon 5. **c** The carboxy terminal 40 amino acids sequence of torsinA of the normal protein, the GAG deletion gene product, and the predicted 18-bp deletion gene product, respectively. The 18-bp deletion deletes the whole consensus sequence of the possible threonine phosphorylation site (TKLD) for casein kinase 2 (CK2) (TKLD) [22]

Clinical and molecular observations to date support the theory that dystonia can be caused by decreased release of dopamine into the striatum during youth [49]. Dystonia/dystonic symptoms can be caused by mutations in enzymes critical to dopamine synthesis, including tyrosine hydroxylase [10, 11], L-aromatic amino acid decarboxylase [50] and GTP cyclohydrolase I, the co-factor regenerating enzyme for tyrosine hydroxylase [8]. Drugs, such as neuroleptics, which block D2 receptors, can also cause dystonic symptoms, especially in younger patients [51, 52]. In line with this theory, highest expression levels of torsinA message in the human brain are found in dopaminergic neurons of the substantia nigra [18, 19]. The death of these same neurons in Parkinson disease and the prevalence of dystonic movements in Parkinson's patients with early onset suggest converging etiologies. Recent studies indicate that Lewy bodies associated with degenerative changes in Parkinson's disease contain torsinA as well as α-synuclein [20, 21], the latter being mutated in some familial forms of Parkinson's disease [53, 54]. The present study also confirms the age-dependent occurrence of dystonic symptoms in Parkinson's disease, with an increased incidence in younger patients [17]. This correlation did not extend to the genetic level in this study, however, in that no mutations in *TOR1A* were found in these EOP patients. The two known mutations in *TOR1A* to date, the GAG and 18-bp deletions, both underlie syndromes of primary early onset dystonia.

Acknowledgements We thank all patients and family members for participating in this study, especially the Fasciana family who has provided extraordinary support without which these studies would not have reached fruition, and Ms. Suzanne McDavitt for skilled preparation of this manuscript. Furthermore, we thank Dr. Fogel (Heidelberg, Germany), Dr. Standaert (Massachusetts General Hospital), and Dr. Jang-Ho Cha (Massachusetts General Hospital) for contribution of patient samples. This work was funded by the Jack Fasciana Fund for Support of Dystonia Research (X.O.B.), the Dystonia Medical Research Foundation (L.O., P.L.K., S.B.B.), the Bachmann-Strauss Dystonia and Parkinson's Foundation (M.B.), the Deutsche Parkinsonvereinigung (H.J. and P.V.), and NINDS grants NS28384 (X.O.B.), NS38142 (L.O.), and NS37409 (X.O.B., L.O., and J.F.). Drs. Klein and Kamm were fellows of the Deutsche Forschungsgemeinschaft.

References

1. Fahn S (1988) Concept and classification of dystonia. Adv Neurol 50:1–8
2. Müller U, Steinberger D, Nemeth AH (1998) Clinical and molecular genetics of primary dystonia. Neurogenetics 1:165–177
3. Klein C, Breakefield XO, Ozelius L (1999) Genetics of primary dystonia. Semin Neurol 3:271–280
4. Dobyns WB, Ozelius LJ, Kramer PL, Brashear A, Farlow MR, Perry TR, Walsh LE, Kasarskis EJ, Butler TJ, Breakefield XO (1993) Rapid-onset dystonia-parkinsonism. Neurology 43: 2596–2602
5. Kramer PL, Mineta M, Klein C, Schilling K, deLeon D, Farlow MR, Breakefield XO, Bressman S, Dobyns WB, Ozelius LJ, Brashear A (1999) Rapid-onset dystonia parkinsonism: linkage to chromosome 19q13. Ann Neurol 46:176–182
6. Segawa M, Hosaka A, Miyagawa F, Nomura Y, Imai H (1976) Hereditary progressive dystonia with marked diurnal fluctuation. Adv Neurol 14:215–233
7. Nygaard T, Wilhelmsen K, Risch N, Brown D, Trugman J, Gilliam C, Fahn S, Weeks DE (1993) Linkage mapping of dopa-responsive dystonia (DRD) to chromosome 14q. Nat Genet 5:386–391
8. Ichinose H, Suzuki T, Inagaki T, Ohye T, Nagatsu T (1999) Molecular genetics of dopa-responsive dystonia. Biol Chem 380:1355–1364
9. Craig SP, Buckle VJ, Lamouroux A, Mallet J, Craig I (1986) Localization of the human tyrosine hydroxylase gene to 11p15: gene duplication and evolution of metabolic pathways. Cytogenet Cell Genet 42:29–32
10. Ludecke B, Dworniczak B, Bartholome K (1995) A point mutation in the tyrosine hydroxylase gene associated with Segawa's syndrome. Hum Genet 95:123–125
11. Bräutigam C, Steenbergen-Spanjers GC, Hoffmann GF, Dionissi-Vici C, Heuvel LP van den, Smeitink JA, Wevers RA (1999) Biochemical and molecular genetic characteristics of the severe form of tyrosine hydroxylase deficiency. Clin Chem 45:2073–2078
12. Lee LV, Kupke KG, Caballar-Gonzaga F, Hebron-Ortiz M, Müller U (1991) The phenotype of the X-linked dystonia-parkinsonism syndrome. An assessment of 42 cases in the Philippines. Medicine (Baltimore) 70:179–187
13. Kupke KG, Lee LV, Müller U (1990) Assignment of the X-linked torsion dystonia gene to Xq21 by linkage analysis. Neurology 40:1438–1442
14. Lücking CB, Durr A, Bonifati V, Vaughan J, De Michele G, Gasser T, Harhangi BS, Meco G, Denefle P, Wood NW, Agid Y, Brice A (2000) Association between early-onset Parkinson's disease and mutations in the parkin gene. French Parkinson's Disease Genetic Study Group. N Engl J Med 342:1560–1567
15. Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Shimizu N, Iwai K, Chiba T, Tanaka K, Suzuki T (2000) Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 25:302–305
16. Klein C, Pramstaller PP, Kis B, Page CC, Kann M, Leung J, Woodward H, Castellan CC, Scherer M, Vieregge P, Breakefield XO, Kramer PL, Ozelius LJ (2000) Parkin deletions in a family with adult-onset, tremor-dominant parkinsonism: expanding the phenotype. Ann Neurol 48:65–71
17. Wagner ML, Fedak MN, Sage JI, Mark MH (1996) Complications of disease and therapy: a comparison younger and older patients with Parkinson's disease. Ann Clin Lab Sci 26:389–395
18. Augood SJ, Penney JB, Friberg I, Breakefield XO, Young A, Ozelius LJ, Standaert DG (1998) Expression of the early-onset torsion dystonia gene (*DYT1*) in human brain. Ann Neurol 43:669–673
19. Augood SJ, Martin DM, Ozelius LJ, Breakefield XO, Penney JBJ, Standaert DG (1999) Distribution of the mRNAs encoding torsinA and torsinB in the adult human brain. Ann Neurol 46:761–769
20. Shashidharan P, Good PF, Hsu A, Perl DP, Brin MF, Olanow CW (2000) TorsinA accumulation in Lewy bodies in sporadic Parkinson's. Brain Res 877:379–381
21. Sharma N, Hewett J, Ozelius LJ, Ramesh V, McLean P, Breakefield XO, Hyman B (2001) A close association of torsinA and alpha synuclein in Lewy bodies: a fluorescence resonance energy transfer study. Am J Pathol (in press)
22. Ozelius LJ, Hewett J, Page C, Bressman S, Kramer P, Shalish C, Leon D, Brin M de, Raymond D, Corey DP, Fahn S, Risch N, Buckler A, Gusella JF, Breakefield XO (1997) The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. Nat Genet 17:40–48
23. Klein C, Friedman J, Bressman S, Vieregge P, Brin MF, Pramstaller PP, Leon D de, Hagenah J, Sieberer M, Fleet C, Kiely R, Xin W, Breakefield XO, Ozelius LJ, Sims KB (1999) Genetic testing for early-onset torsion dystonia (DYT1): introduction of a simple screening method, experiences from testing of a large patient cohort, and ethical aspects. Genet Test 3:323–328

24. Bressman SB, Sabatti C, Raymond D, De Leon D, Klein C, Kramer PL, Brin MF, Fahn S, Breakefield XO, Ozelius LJ, Risch NJ (2000) The DYT1 phenotype and guidelines for diagnostic testing. Neurology 54:1746–1752
25. Risch NJ, Bressman SB, deLeon D, Brin MF, Burke RE, Greene PE, Shale H, Claus EB, Cupples LA, Fahn S (1990) Segregation analysis of idiopathic torsion dystonia in Ashkenazi Jews suggests autosomal dominant inheritance. Am J Hum Genet 46:533–538
26. Ozelius LJ, Page CE, Klein C, Hewett JW, Mineta M, Leung J, Shalish C, Bressman SB, Leon D de, Brin MF, Fahn S, Corey DP, Breakefield XO (1999) The *TOR1A* (*DYT1*) gene family and its role in early onset torsion dystonia. Genomics 62:377–384
27. Kamm C, Castelon-Konkiewitz E, Naumann M, Heinen F, Brack M, Nebe A, Ceballos-Baumann A, Gasser T (1999) GAG deletion in the *DYT1* gene in early limb-onset idiopathic torsion dystonia in Germany. Mov Disord 14:681–683
28. Brassat D, Camuzat A, Vidailhet M, Feki I, Jedynak P, Klap P, Agid Y, Dürr A, Brice A (2000) Frequency of the *DYT1* mutation in primary dystonia without family history. Arch Neurol 57:333–335
29. Valente EM, Warner TT, Harman PR, Mathen D, Fletcher NA, Marsden CD, Bhatia KP, Wood NW (1998) The role of DYT1 in primary torsion dystonia in Europe. Brain 121:2335–2339
30. Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879
31. Neuhuber F, Radacher M, Sorgo G (1996) Analysis of STR-PCR products with high-resolution denaturing PAGE. Int J Legal Med 108:225–226
32. Deimling A von, Bender B, Louis DN, Wiestler OD (1993) A rapid and non-radioactive assay for the detection of allelic loss in human gliomas. Neuropathol Appl Neurobiol 19:524–529
33. Jacobs H, Vieregge A, Vieregge P (2000) Sexuality in young patients with Parkinson's disease: a population-based comparison with healthy controls. J Neurol Neurosurg Psychiatry 69: 550–552
34. Ozelius LJ, Klein C, Friedman J, Kann M, Jacobs H, Pramstaller PP, Breakefield XO, Vieregge P (1999) Clinical and genetic evaluation of twelve patients with young-onset Parkinson's disease and report of new mutation in the parkin gene. Neurology 52 [Suppl 2]: AII
35. Wu Q, Krianer AR (1996) U-I mediated exon definition interactions between AT-AC and GT-AG introns. Science 274:1005–1008
36. Neuwald AF, Aravind L, Spouge JL, Koonin EV (1999) AAA+: a class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. Genome Res 9:27–43
37. Hewett J, Gonzalez-Agosti C, Slater D, Li S, Ziefer P, Bergeron D, Jacoby DJ, Ozelius LJ, Ramesh V, Breakefield XO (2000) Mutant torsinA, responsible for early onset torsion dystonia, forms membrane inclusions in cultured neural cells. Hum Mol Genet 22:1403–1413
38. Klein C, Brin MF, deLeon D, Limborska SA, Ivanova-Smolenskaya IA, Bressman SB, Friedman A, Markova ED, Risch NJ, Breakefield XO, Ozelius LJ (1998) De novo mutations (GAG deletion) in the *DYT1* gene in two non-Jewish patients with early-onset dystonia. Hum Mol Genet 7:1133–1136
39. Kustedjo K, Bracey MH, Cravatt BF (2000) TorsinA and its torsion dystonia-associated mutant forms are lumenal glycoproteins that exhibit distinct subcellular localizations. J Biol Chem 275:27933–27939
40. Zeman W, Dyken P (1967) Dystonia musculorum deformans: clinical, genetic and patho-anatomical studies. Psychiatr Neurol Neurochir 10:77–121
41. Hedreen JC, Zweig RM, DeLong MR, Whitehouse PJ, Price DL (1988) Primary dystonias: a review of the pathology and suggestions for new directions of study. Adv Neurol 50:123–132
42. Leube B, Kessler KR, Ferbert A, Ebke M, Schwendemann G, Erbguth F, Benecke R, Auburger G (1999) Phenotypic variability of the DYT1 mutation in German dystonia patients. Acta Neurol Scand 99:248–251
43. Gasser T, Windgassen K, Bereznai B, Kabus C, Ludolph AC (1998) Phenotypic expression of the DYT1 mutation: a family with writer's cramp of juvenile onset. Ann Neurol 44:126–128
44. Quinn NP (1996) Essential myoclonus and myoclonic dystonia. Mov Disord 11:119–124
45. Gasser T (1998) Inherited myoclonus-dystonia syndrome. Adv Neurol 78:325–334
46. Nygaard TG, Raymond D, Chen C, Nishino I, Greene PE, Jennings D, Heiman GA, Klein C, Saunders-Pullman RJ, Kramer P, Ozelius LJ, Bressman SB (1999) Localization of a gene for myoclonus-dystonia to chromosome 7q21-q31. Ann Neurol 46:794–798
47. Klein C, Schilling K, Saunders-Pullman RJ, Garrels J, Breakefield XO, Brin MF, deLeon D, Doheny D, Fahn S, Fink S, Forsgren L, Friedmna J, Frucht S, Harris J, Holmgren G, Kis B, Kurlan R, Kyllerman M, Lang A, Leung J, Raymond D, Robishaw J, Sanner G, Schwinger E, Tabamo R, Tagliati M, Vieregge P, Wahlstrom J, Wendt K, Kramer P, Bressman S, Ozelius LJ (2000) A major locus for myoclonus-dystonia maps to chromosome 7q in eight families. Amer J Hum Genet 67:1314–1319
48. Klein C, Brin MF, Kramer P, Sena-Esteves M, Leon D de, Doheny D, Bressman S, Fahn S, Breakefield XO, Ozelius LJ (1999) Association of a missense change in the D2 dopamine receptor with myoclonus dystonia. Proc Natl Acad Sci USA 27:5173–5176
49. Ozelius LJ, Hewett JW, Page CE, Bressman SB, Kramer PL, Shalish C, deLeon D, Brin M, Raymond D, Jacoby D, Penney J, Risch NJ, Fahn S, Gusella JF, Breakefield XO (1998) The gene (*DYT1*) for early onset torsion dystonia encodes a novel protein related to the Clp protease/heat shock family. In: Fahn S, Marsden CD, DeLong M (eds) Dystonia 3, vol. 78. Lippincott-Raven, Philadelphia, pp 93–105
50. Swoboda KJ, Hyland K, Goldstein DS, Kuban KC, Arnold LA, Holmes CS, Levy HL (1999) Clinical and therapeutic observations in aromatic L-amino acid decarboxylase deficiency. Neurology 53:1205–1211
51. Burke RE, Fahn S, Jankovic J, Marsden CD, Lang AE, Gollop S, Ilson J (1982) Tardive dystonia: late onset persistent dystonia caused by antipsychotic drugs. Neurology 32:1335–1346
52. LeWitt PA (1995) Dystonia caused by drugs. In: King J, Tsui C, Calne DB (eds) Handbook of dystonia. Dekker, New York, pp 227–240
53. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science 276: 2045–2047
54. Krüger R, Kuhn W, Müller T, Woitalla D, Graeger M, Kösel S, Przuntek H, Epplen JT, Schols L, Riess O (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 18:106–108