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The TOR1A (DYT1) Gene Family and Its Role in Early Onset Torsion Dystonia

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Most cases of early onset torsion dystonia are caused by a 3-bp deletion (GAG) in the coding region of the TOR1A gene (alias DYT1, DQ2), resulting in loss of a glutamic acid in the carboxy terminal of the encoded protein, torsin A. TOR1A and its homologue TOR1B (alias DQ1) are located adjacent to each other on human chromosome 9q34. Both genes comprise five similar exons; each gene spans a 10-kb region. Mutational analysis of most of the coding region and splice junctions of TOR1A and TOR1B did not reveal additional mutations in typical early onset cases lacking the GAG deletion ($N = 17$), in dystonic individuals with apparent homozygosity in the 9q34 chromosomal region ($N = 5$), or in a representative Ashkenazic Jewish individual with late onset dystonia, who shared a common haplotype in the 9q34 region with other late onset individuals in this ethnic group. A database search revealed a family of nine related genes (50–70% similarity) and their orthologues in species including human, mouse, rat, pig, zebrafish, fruitfly, and nematode. At least four of these genes occur in the human genome. Proteins encoded by this gene family share functional domains with the AAA/HSP/Clp-ATPase superfamily of chaperone-like proteins, but appear to represent a distinct evolutionary branch.

INTRODUCTION

Dystonia is a movement disorder characterized by sustained muscle contractions, frequently causing twisting and repetitive movements or abnormal postures (Fahn *et al.*, 1998). Over 12 different gene loci have been implicated in various forms of primary, hereditary dystonia (Mueller *et al.*, 1998; Klein *et al.*, in press, b). The TOR1A (DYT1) locus on human chromosome 9q34 is responsible for the most severe form of hereditary dystonia—early onset, generalized torsion dystonia (Ozelius *et al.*, 1989; Kramer *et al.*, 1994). In this form, symptoms usually begin in a limb at a mean age of 12.5 years, with progression, in most cases, to involve multiple body parts over a subsequent 5-year period (Bressman *et al.*, 1994). Inheritance follows an autosomal dominant mode of transmission with reduced penetrance (30–40%; Risch *et al.*, 1990). The TOR1A gene on human chromosome 9q34 (Ozelius *et al.*, 1989) was identified and determined to encode a novel protein, torsin A (Ozelius *et al.*, 1997b). A single mutation, a 3-bp (GAG) deletion, in the heterozygous state accounts for 50–60% of non-Jewish (NJ) individuals and over 90% of Ashkenazi Jewish (AJ) individuals with this form of dystonia (Bressman *et al.*, 1994; Klein *et al.*, in press, a). The predominance of this mutation may reflect both a unique phenotype produced by the altered protein, with loss of one of a pair of glutamic acids in the carboxy-terminal region, and an increased frequency of this mutation, due to genetic instability in an imperfect tandem 24-bp repeat in the region of the deletion (Klein *et al.*, 1998).

The TOR1A gene is expressed selectively in specific regions of the human brain, with highest levels in the dopaminergic neurons of the substantia nigra (Augood *et al.*, 1998, 1999). The involvement of these neurons in the etiology of dystonia is consistent with other genetic,

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pharmacologic, and clinical studies. Metabolic deficiency states that compromise dopamine synthesis can also manifest with severe childhood-onset dystonia, including mutations in genes for GTP-cyclohydrolase I (Ichinose *et al.*, 1994) and tyrosine hydroxylase (Ludecke *et al.*, 1995; Knappskog *et al.*, 1995; Wevers *et al.*, 1999) underlying dopa-responsive dystonia and in genes for L-aromatic amino acid decarboxylase (Hyland *et al.*, 1998) and dihydropteridine reductase (Blau *et al.*, 1998) in other neurologic syndromes with marked dystonic features. Dystonic symptoms can also be elicited by D2 receptor blockers (LeWitt, 1995), and a missense mutation in the D2 receptor has been implicated in a family with myoclonus dystonia (Klein *et al.*, 1999).

The TOR1A gene encoding torsin A is a member of a larger gene family, including an adjacent gene, TOR1B (DQ1), which encodes a highly homologous (70%) protein, torsin B. The human genome contains at least two other related genes, TOR2A and TOR3A (TORP1 and TORP2; Ozelius *et al.*, 1997b). All four human genes are expressed in most fetal and adult tissues. The interest in the TOR1A gene family derives not only from its role in a neurologic disease, but also from the similarity of the deduced protein, torsin A, to a superfamily of proteins with a variety of chaperone-like functions (Ozelius *et al.*, 1998). The similarity includes an ATP-binding domain and 11 conserved motifs and predicted secondary structure (Neuwald *et al.*, 1999; Ozelius *et al.*, 1997b; Lupas *et al.*, 1997) shared with the heat shock protein (HSP)/Clp ATPase and the "ATPase associated with a variety of cellular activities" (AAA) superfamily (Parsell and Lindquist, 1993; Gottesman *et al.*, 1997; Confalonieri and Duguet, 1995). This AAA+ family includes the *N*-ethylmaleimide-sensitive fusion (NSF) protein, which has been implicated in vesicle fusion and neurotransmitter release (Hanson *et al.*, 1997; Haas, 1998), and HSP104, which has been shown to determine the state of prion aggregation (DebBurman *et al.*, 1997).

This study documents the exon structure and physical map of the human TOR1A and TOR1B genes. These genes were screened for mutations using intronic and flanking primers to amplify and scan genomic DNA from three types of dystonia patients: those with early onset, generalized dystonia who lack the GAG-deletion; those who appear to be homozygous in this 9q34 chromosomal region; and a representative AJ individual with late onset dystonia, who shared a common haplotype of polymorphic alleles in this region with other late onset AJ individuals, suggesting a founder mutation. This study also elucidates the extended TOR1A gene family in a variety of species and clarifies its relationship to the AAA+ family.

MATERIALS AND METHODS

Clinical criteria and patient samples. Clinical criteria and patient samples from affected individuals were obtained through the Movement Disorders Groups at Columbia Presbyterian Medical Center, Mount Sinai School of Medicine, and Beth Israel Hospital (New York) under IRB-approved, informed consent protocols. The criteria for the diagnosis of primary torsion dystonia and the methods of evaluation were the same as described previously (Bressman *et al.*, 1994).

Analysis of gene structure. DNA was processed according to standard methods (Sambrook et al., 1989). Cosmids spanning the TOR1A region (Ozelius *et al.*, 1997a) were sequenced directly using primers spaced throughout the cDNAs (DQ1 and DQ2; Ozelius *et al.*, 1997b). The resultant genomic sequence was aligned with the cDNA sequence revealing the exon–intron boundaries. The size of the introns was determined by amplifying across the introns using exonic primers with Elongase enzyme and 1.8 mM Mg^{2+} when possible, according to the manufacturer's instructions (Life Technologies).

Dideoxy cycle sequencing. Dideoxy cycle sequencing was performed with the Perkin–Elmer AmpliSequence Sequencing kit (Perkin–Elmer) using specific primers labeled with [α-^{33}P]dATP (2000 Ci/mmol; NEN). Direct cycle sequencing (step 1, 95°C for 2 min; step 2, 95°C for 1 min; step 3, 60°C for 1 min; step 4, 72°C for 1 min; cycle steps 2–4 $\times$25; step 5, 4°C for 5 min) was performed after enzymatic clean-up with exonuclease I and shrimp alkaline phosphatase (USB) for 15 min at 37°C and 15 min at 85°C.

After DNA extraction from whole blood or lymphoblastoid lines following standard protocols, PCR amplification was performed using primers 6418 and 6419 spanning the critical region of the TOR1A gene (Ozelius *et al.*, 1997b). PCR products were resolved on a denaturing 6% polyacrylamide gel and visualized by silver staining (Deimling *et al.*, 1993; Klein *et al.*, in press, a).

Database analysis. The amino acid sequences of torsin A and torsin B were used to search the database of expressed sequence tags (dbEST) to identify additional homologues and orthologues. ESTs were grouped by nucleotide sequence and used to search dbEST iteratively. In seven cases, UniGene clusters that represent homologues were identified. ESTs were assembled into contigs with Sequencher (GeneCodes) or with the EST Assembly Machine and EST Extractor at TIGEM (http://gcg.tigem.it/cgi-bin/uniestass.pl; http://gcg.tigem.it/blastextract/estextract.html). Amino acid sequences of EST contigs were predicted from open reading frames with similarity to torsin A and torsin B. The *Caenorhabditis elegans* database (http://www.sanger.ac.uk/Projects/C_elegans/blast_server.shtml) was searched to identify predicted proteins from the *C. elegans* genome project. Gene designations were approved by the Human and Mouse Naming Committees.

Phylogenetic relationships were determined by alignment of the predicted amino acid sequences of torsins, torps, and heat-shock proteins using ClustalX (Thompson *et al.*, 1994). The tree was calculated by ClustalX with 1000 bootstrap trials, excluding positions with gaps and correcting for multiple substitutions, and was displayed for plotting with TreeView 1.5 (http://taxonomy.zoology.gla.ac.uk/rod/rod.html).

Secondary structure predictions were made using the Protein Sequence Analysis Server at Boston University (http://bmerc-www.bu.edu/psa/).

RESULTS

Exon Structure of TOR1A and TOR1B

Human TOR1A and TOR1B transcripts (DYT1 or DQ2, and DQ1, respectively) were found previously to be highly similar (71% identical at the amino acid level) and encoded by adjacent genes on chromosome 9q34, which face in opposite orientations with their 3′ ends as nearest neighbors (Ozelius *et al.*, 1997a,b). Each gene has five exons spanning about 10 kb, with the same location of splice sites; these two genes are

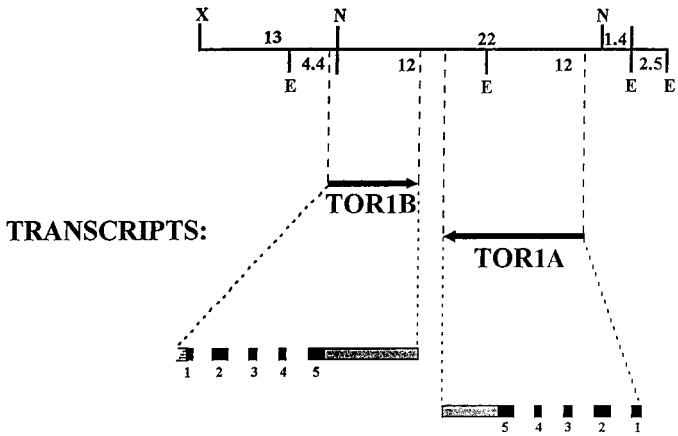

FIG. 1. Exon structure and orientation in chromosome. (**Top**) The restriction map of this genomic region: restriction enzymes, X, *Xba*I; E, *Eco*RI; N, *Not*I; and fragment sites (Ozelius *et al.*, 1997a). (**Middle**) The extent and orientation of TOR1B and TOR1A genes (arrows) are shown relative to the restriction map of this genomic region. (**Bottom**) The exon structures are depicted as exons in boxes with coding regions in solid boxes and untranslated regions in shaded boxes. The full extent of exon 1 has not been determined for either gene.

separated from each other in the genome by about 2 kb (Fig. 1). The size of the exons and introns of these genes and the nucleotide sequences near the intron–exon junctions are listed in Table 1.

Screen for Mutations in Dystonic Individuals

Based on the hypothesis that other mutations, in addition to the GAG deletion, occur in the TOR1A gene and that at least some of these may cause dystonia, a mutational screen of coding sequences was undertaken for TOR1A and TOR1B for three groups of dystonia patients (total 23). All affected individuals tested negative for the GAG deletion. The first group consisted of 17 patients (5 of AJ descent and 12 non-Jewish) with features typical of early onset dystonia, including limb onset from 3 to 19 years of age with spread to involve at least one and frequently multiple body parts (Table 2). The second group comprised 5 patients (4 AJ and 1 AJ/NJ) with early to midlife onset (4 to 35 years) whose haplotype in the TOR1A region appeared homozygous. This homozygosity could have resulted from consanguinity or deletion of genomic DNA in this region on one chromosome, for some or all of the polymorphic markers—D9S2160, D9S2161, D9S63, D9S2162, in a ≤320-kb region, including the TOR1A locus (Ozelius *et al.*, 1997a; Table 3). The third group was represented by one individual from a set of 11, out of 88 AJ patients tested with late onset, focal dystonia, who carried the same haplotype for nine polymorphic markers (D9S62a, D9S62b, D9S159, D9S2158, D9S2159, D9S2160, D9S63, D9S2162, and ASS), which span the TOR1A region. Age of onset in these 11 patients ranged from 29 to 66 years (mean age 48 years) with focal symptoms involving neck (3), larynx (2), upper face (4), or arm (2). Phasing of these chromosomes was possible in one family with two affected individuals confirmed with a common haplotype.

Genomic DNA from these 23 patients was used to amplify exons from TOR1A and TOR1B with the flank-

TABLE 1

Exon–Intron Structure of TOR1A and TOR1B Genes

Exon	Size (bp)	3′ end of exon	5′ splice site	Intron (kb)[a]	3′ splice site	5′ end of exon	Exon
				DYTI			
1	178	. . .CGG GAG G R E A	GTAGGCTGGG. . .	1.2	. . .TCTTTCCCAG	CA CTG CAG AAG. . . L G K	2
2	266	. . .TTG TAC AAG L Y K	GCAAGGATGG. . .	1.5	. . .TTTAATTCAG	GAT CAG TTA. . . D Q L	3
3	176	. . .TTT CTC AG F L S	GTAAGGTCAG. . .	0.097	. . .TGTTTTGCAG	C AAT GCT GGA. . . N A G	4
4	128	. . .AAG AAC AG K N S	GTGAGTAGGG. . .	4	. . .TTCTTCCCAG	T GGC TTC TGG. . . G F W	5
5	251	. . .GAT GAT TGA D D —					
				TORB			
1	n.d.	. . .GCT TCG G A S A		n.d.	. . .GTTCTTGCAG	CT CTC AAG CTG. . . L K L	2
2	266	. . .CTG TAC CAG L Y Q	GCAAGAGAAC. . .	3	. . .GTTGGTCCAG	GAC CAG TTA. . . D Q L	3
3	176	. . .TTT CTC AG F L S	GTCAGCGGGA. . .	1.8	. . .GCAAACTCAG	C AAT GCA GGC. . . N A G	4
4	128	. . .AAA CAC AG K H S	GTGAGTCCAC. . .	.31	. . .TGTTCTGCAG	T GGC CTG TGG. . . G L W	5
5	242	. . .TTC CAC TGA F H —					

[a] Sizes of introns were approximated by gel resolution of PCR products.

TABLE 2

Non-GAG Deleted Dystonia Patients Evaluated for Mutations in TOR1A and TOR1B

Type	Number of individuals	Age of onset (years)	Site of onset[a] (No.)	Sites involved (No.)
Early onset				
AJ	5	6, 8, 8, 10, 19	A(4), ANL(1)	A(7), N(3), T(1), L(2)
NJ	12	3, 3, 6, 8, 9, 10, 13, 14(2), 16, 11, 13, 14, 14, 16, 18	A(9), AU(1), G(1), L(1)	A(20), G(9), R(6), K(4), U(4), L(4), N(6), P(1), S(1), T(2), F(2), J(3), U(2)
Potential homozygosity				
AJ[b]	5	4, 6, 7, 26, 35	L(4), M(1)	L(4), M(1), N(2), P(1), T(1), U(1), H(2)
Late onset				
AJ	1	66	U	U

[a] Body sites: U, upper face; F, lower face; J, jaw; T, tongue; P, pharynx; L, larynx; S, speech, swallowing; A, arm; K, trunk; G, leg; N, neck; H, hearing loss. No., number of individuals in group with that site affected.
[b] Three of these individuals had all known AJ ancestors; one was 7/8 AJ, and one was 1/2 AJ.

ing intronic primers listed in Table 4. A screen of known coding sequence in the five exons of TOR1A (excluding the 40 bp of the coding region and the 5′ splice junction of exon 1, as this region is very G-C-rich and proved difficult to amplify consistently) and exons 2–5 of TOR1B (again the region around the 5′ end of intron 1 was difficult to PCR) did not reveal any new changes that would alter the amino acid sequence of the protein or splicing of the messages. A number of previously described polymorphisms were observed (Ozelius *et al.*, 1997a). None of the five apparently homozygous patients was heterozygous at any of three polymorphisms in the TOR1A gene (Ozelius *et al.*, 1997b), leaving open potential homozygosity or hemizygosity due to a chromosomal deletion.

A Gene Family of Torsins and Torsin-Related Proteins in Different Species

We used the sequences of torsin A and torsin B to search databases for orthologues and homologues in other species. Mouse orthologues of both genes are represented by ESTs, which are clustered in the UniGene database. Pig and rat orthologues for torsin A are also present in the EST database. We also identified homologues in mammals, fish, flies, and nematodes (see Table 5). A phylogenetic analysis of the predicted protein sequences (Fig. 2) indicated that one of these—an EST from zebrafish—is closely related to torsin A and torsin B, but not sufficiently similar to represent an orthologue and so is termed torsin C. Other homologues are more distant from the torsins and so are termed torsin-related proteins, or torps.

TABLE 3

Genotypes of Dystonic Individuals with Apparent Homozygosity in the TOR1A Region

Sample number	Genotype			
	D9S2160[a]	D9S2161[b]	D9S63	D9S2162
18843	3,4	2,2	8,8	4,4
13709	4,4	2,6	14,14	NT[c]
14122[d]	3,3	4,4	0,0	5,5
13945	4,4	1,5	18,18	NT[c]
14040	2,5	1,2	16,16	2,4

[a] Markers listed from centromere to telomere: D9S2160–<40 kb–D9S2161–150 kb–D9S63–130 kb–D9S2162 (Ozelius *et al.*, 1997a).
[b] Position of TOR1A gene.
[c] Not tested.
[d] This individual had onset at 7 years in the arm with eventual involvement of the arm and neck and a positive family history of movement disorder (father with tremor). He was also apparently homozygous for markers D9S159–D9S2158–D9S2159, which are proximal to DS2160 (total region of 100 kb).

TABLE 4

Primers Used to Amplify TOR1A and TOR1B Genes

Exon	Primer sequence (5′ → 3′)	Product size (bp)[a]
	Intron primers used to amplify DYT1 exons	
1	GCAAAACAGGGCTTTGTACCG	
	AGTAGAGACGCGGGTAGATG	
	GCGTCTCTACTGCCTCTTCG	
	ATGCCCTGGTCCTAGTTCAG	
2	GGTTTCGCAAGGTGCTTGGA	408
	GGGATTCCAAACTTCCATCC	
3 and 4	TCCATGGGGTTGGTAGGAAC	804
	GGTGACAGAGTAAAACTATCTG	
5	GACCCCCAGTAGACGTTTGT	640
	GTAAAAAATCATGAGCCCTGC	
	Intron primers used to amplify TORB exons	
1	n.d.[b]	—
2	CCAGAGTTAGTGAGCAGGTC	526
	GAAGCGTTAAGGACCTCCAC	
3	ATCTATCTCTGCCAATTTCCAC	466
	GTCCTGGTAAACAAAGTGCTG	
4	TGGGGTTACTCTATGTTGGTC	440
	CTAGCACAGTATGCCCTAAG	
5	TGAGGAATGTGCTGAGGGTC	333
	GCTGTCTCCTACCCCATCTG	

[a] PCR products were generated using oligonucleotides synthesized from intronic sequences, and accordingly the size of each product includes both intron and exon sequence.
[b] Not done. It was not possible to identify primers that could consistently PCR this exon.

TABLE 5

GenBank Sequences Representing the Torsin Gene Family

Protein	Organism	Gene	Alias	Locus	Accession No.	UniGene
hTorsin A	Human	*TOR1A*	DYT1, DQ2	Chromosome 9, D9S159–D9S164	AF007871	Hs.19261
mTorsin A	Mouse	*Tor1a*			AA230756	Mm.40438
rTorsin A	Rat				AA850233	Rn.20041
sTorsin A	Pig				Au058534	
hTorsin B	Human	*TOR1B*	DQ1	Chromosome 9, D9S159–D9S164	AF007872	Hs.5091
mTorsin B	Mouse	*Tor1b*			AA596988	
drTorsin C	Zebrafish	*TOR1C*			AA542632	
hTorp2a	Human	*TOR2A*			AA873275	Hs.59038
mTorp2a	Mouse	*Tor2a*			AA981789	Mm.33875
rTorp2a	Rat				H31561	
hTorp3a	Human	*TOR3A*			AA150869	Hs.26267
mTorp3a	Mouse	*Tor3a*			AA791729	
dmTorp4a	Fruitfly	EG:84H4.1		DMC84H4	AL031766	
ceTorp5a	Nematode	F44G4.1		CEC18E9	P54073	
ceTorp5b	Nematode	Y37A1B.12		CEY37A1B	AL023835	
ceTorp5c	Nematode	Y37A1B.13		CEY37A1B	AL023835	

Note. Proteins are named torsins or torps according to phylogenetic relationships as determined by ClustalX and shown in Fig. 2. Species are denoted for orthologs as follows: h, *Homo sapiens;* m, *Mus musculus;* r, *Rattus norvegicus;* dr, *Danio rerio;* dm, *Drosophila melanogaster;* ce, *Caenorhabditis elegans.* Gene names have been approved by the Mouse and Human Gene Nomenclature Committees. Accession numbers indicate either a GenBank entry for a cloned gene or a representative EST.

Torp2 and torp3 are expressed by both mouse and human. Human ESTs for both torp2 and torp3 and mouse ESTs for torp2 are clustered in the UniGene database, which facilitated assembly of cDNA sequence (see Table 5). Torp4 is expressed by *Drosophila melanogaster,* but apparently not by other species. The three torps expressed by *C. elegans* are more related to one another than to other members of the family and so together are denoted torp5, with subtypes a, b, and c. Since the *C. elegans* genome project is largely complete, there are probably not additional members in nematodes. An apparent pseudogene for TOR1B is located on human chromosome 2p13 with several BAC clones from this region showing highly homologous sequences; however, there is no open reading frame of any length among these sequences.

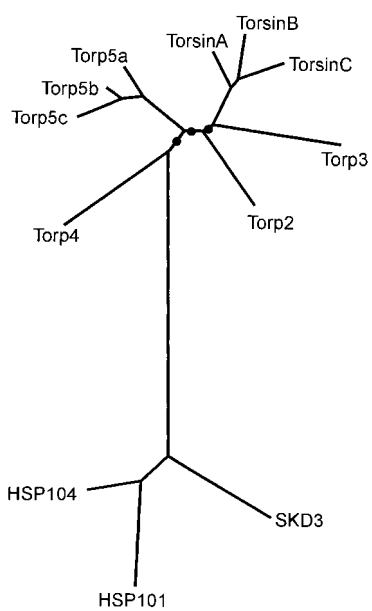

FIG. 2. Phylogenetic relationships among the Torsin family and relationship to the HSP101 family. For each protein, the orthologue for which there was the longest sequence was used for the phylogeny analysis. Orthologues, when included, are represented by much shorter branches than those separating homologues (not shown). Branches marked by dots are not statistically significant.

We included in the phylogenetic analysis some members of the HSP101 superfamily of heat-shock proteins, representing both class 1 and class 2 subtypes (Parsell and Lindquist, 1993). These clearly group together, but far from the torsins and torps. Thus the Torsin family of proteins is not simply a branch of the HSP101 family, but is a separate group that shares some characteristic domains (Ozelius *et al.*, 1997b).

DISCUSSION

The TOR1A (DYT1) gene for early onset torsion dystonia is remarkable in two ways: the large majority of patients with this syndrome bear the same mutation (GAG deletion) in the heterozygous state (Ozelius *et al.*, 1997b; Klein *et al.*, in press, a), and identification of this gene reveals a novel family of proteins with features of the heat-shock/chaperone and AAA+ superfamily (Ozelius *et al.*, 1997b; Neuwald *et al.*, 1999). The present study defines the exon structure of this gene and its adjacent homologue, TOR1B. A screen for additional mutations in the TOR1A and the TOR1B loci in several types of dystonia patients with features consistent with TOR1A involvement did not reveal additional mutations. The inability to identify additional mutations in the TOR1A gene suggests that the struc-

tural change in the torsin A protein caused by the GAG deletion (loss of a glutamic acid residue in the carboxy terminal) may be functionally unique. While a phylogenetic analysis of the TOR1A gene family showed it to represent a distinct branch related to the AAA+ (HSP/Clp-ATPase–AAA) superfamily, a detailed sequence comparison between the torsin family members and this superfamily has revealed shared functional and configurational domains (Ozelius *et al.*, 1997b; Neuwald *et al.*, 1999). This superfamily is typified by hexameric architecture, protein–protein interactions, and conformational changes mediated by ATP hydrolysis, and its members mediate a wide range of cellular functions. The function of torsin A remains to be determined, but the high conservation of this family among species suggests an important role in cell biology.

Dominantly inherited conditions, such as early onset dystonia, can result from mutant forms of a protein that either act in a "gain of function" manner unrelated to the functions of the normal protein counterpart, e.g., in triplet repeat diseases where extended glutamine residues cause intracellular precipitation of proteins (Paulson and Fishbeck, 1996; Ross, 1997; DiFiglia *et al.*, 1997), or in a "dominant negative" manner, blocking normal functions of oligomeric protein complexes, such that even a minority of mutant subunits in the complex can disrupt its function, e.g., a missense mutation in one subunit of a K^+ channel that causes deafness (Kubisch *et al.*, 1999). This latter mechanism seems intriguing for dystonia, as members of the AAA+ family typically form six-member, homo-oligomeric ring structures, which interact with one or more other proteins, frequently with substrate proteins or nucleic acids "threaded" through the hole in the ring. The recently described crystal structure of NSF (Lenzen *et al.*, 1998; Neuwald, 1999; Yu *et al.*, 1998)—a member of the AAA+ family involved in vesicle fusion (Whiteheart *et al.*, 1994)—and alignment of torsin A with its structural domains suggest that the region of the GAG deletion in torsin A may be involved either in the formation of the ring structure or in the interaction with partner proteins (D. Corey, unpublished observations). Mutations in the carboxy-terminal portion of some of the HSP proteins, e.g., HS1U and filamentation temperature-sensitive protein, can block association with the partner proteins in a dominant manner, thereby inhibiting proteolytic function of the complex (Missiakas *et al,* 1996; Akiyama *et al,* 1994). By analogy, the GAG deletion in the carboxy region of torsin A could prevent function of an oligomeric complex, even if only a few subunits of a heteromeric torsin A ring bear the mutation. Thus, in the heterozygous condition, with expression of normal and mutant (GAG-deleted) forms of torsin A, the function of the complex might be compromised. Other mutations in torsin A present in the heterozygous state might not disrupt function to the same extent and thus might lead to a milder or no phenotype. However, disruptive mutations present in both alleles of torsin A might cause dystonia or another type of disease.

Based on the above hypotheses, three possible outcomes of other mutations in TOR1A were considered. First, any mutation that disrupts the function of the oligomeric complex might lead to a syndrome of dominantly inherited, early onset dystonia. Presumably only a limited number of changes in the structure of torsin A could produce a dominant-negative effect, and some of these might be present in those early onset, generalized dystonia patients who do not bear the GAG deletion. To test this possibility, we selected a set of AJ and NJ patients without the GAG deletion who had early onset dystonia (<19 years) that began in a limb and progressed to involve at least one other body part (not excluding cranial involvement). Second, if disruption of torsin A function causes dystonia, then null mutations in both alleles of the TOR1A gene might also produce a dystonic syndrome. In the course of haplotype analysis in dystonia cases, a number of individuals were identified who were apparently homozygous for alleles in and around the TOR1A locus and thus might bear a disruptive mutation in one TOR1A allele and deletion of the other allele. Third, some cases of late onset/focal dystonia might be caused by dominantly acting, but less disruptive mutations in the TOR1A gene. This last hypothesis was supported by the finding that 11/88 AJ patients with late onset dystonia appeared to share a common haplotype in the TOR1A region (Ozelius, 1994), suggesting a possible founder mutation with a milder phenotype. Sequence analysis of coding regions of TOR1A and TOR1B genes in genomic DNA from these three types of affected individuals, however, revealed no further mutations in TOR1A nor any in TOR1B. It remains possible that mutations in noncoding sequences in the 5′ flanking region or introns of these genes can disrupt transcription, processing, or stability of these messages or that the GAG deletion confers a novel, deleterious property to torsin A. These patients may also have mutations in one of the 12 or more other human gene loci implicated in primary dystonia (Mueller *et al.*, 1998; Klein *et al.*, in press, b), including other members of the TOR1A gene family or loci encoding nonhomologous proteins that interact functionally with torsin A. To date, however, all multiplex families with early onset dystonia with linkage to 9q34 have the GAG deletion. Further, some patients manifesting apparent homozygosity in this chromosomal region may be hemizygous for TOR1A and/or TOR1B genes with a consequent haploinsufficiency state.

The present study provides the foundation for further analysis of the torsin A gene family and its role in human disease. The elucidation of gene structure of TOR1A and TOR1B and the description of intronic primers for PCR will allow the search for mutations to continue at the genomic level. The elucidation of a cross-species and multimember gene family should aid in defining other syndromes mediated by this protein

family. The features of torsin A shared with the HSP/Clp-ATPase and AAA superfamily and the implication of compromised dopaminergic function in dystonia provide clues as to the function of this new class of proteins in neurons and a link to other movement disorders.

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