Mutations in THAP1 (DYT6) in early-onset dystonia: a genetic screening study

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Summary

Background Mutations in THAP1 were recently identified as the cause of DYT6 primary dystonia; a founder mutation was detected in Amish–Mennonite families, and a different mutation was identified in another family of European descent. To assess more broadly the role of this gene, we screened for mutations in families that included one family member who had early-onset, non-focal primary dystonia.

Methods We identified 36 non-DYT1 multiplex families in which at least one person had non-focal involvement at an age of onset that was younger than 22 years. All three coding exons of THAP1 were sequenced, and the clinical features of individuals with mutations were compared with those of individuals who were negative for mutations in THAP1. Genotype–phenotype differences were also assessed.

Findings Of 36 families, nine (25%) had members with mutations in THAP1, and most were of German, Irish, or Italian ancestry. One family had the Amish–Mennonite founder mutation, whereas the other eight families each had novel, potentially truncating or missense mutations. The clinical features of the families with mutations conformed to the previously described DYT6 phenotype; however, age at onset was extended from 38 years to 49 years. Compared with non-carriers, mutation carriers were younger at onset and their dystonia was more likely to begin in brachial, rather than cervical, muscles, became generalised, and include speech involvement. Genotype–phenotype differences were not found.

Interpretation Mutations in THAP1 underlie a substantial proportion of early-onset primary dystonia in non-DYT1 families. The clinical features that are characteristic of affected individuals who have mutations in THAP1 include limb and cranial muscle involvement, and speech is often affected.

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Introduction The clinical spectrum of primary dystonia is wide, ranging from childhood-onset disease that often generalises, to adult-onset, localised contractions that commonly affect the cervical or cranial muscles. From the earliest descriptions of dystonia, more than 100 years ago, genetic causes were suspected, particularly in individuals who had early onset. Over the past 20 years, five loci (DYT1, DYT6, DYT7, DYT13, and DYT17) have been mapped by genetic linkage in families with primary, pure forms of dystonia; however, the genes for only two, DYT1 and DYT6, have been identified, and the gene for DYT6 was identified only recently. DYT1 is a major cause of early limb-onset disease, whereas DYT6, similar to other mapped loci, was thought to be of limited importance and found in only Amish–Mennonite families who share a founder haplotype. Now that THAP1, the gene that encodes THAP1 (thanatos-associated protein [THAP] domain-containing apoptosis-associated protein 1), has been identified as the gene that is mutated in DYT6, its role can be directly assessed.

We found two different heterozygous mutations in THAP1 in five families. Four of the five families had the same five base pair insertion/three base pair deletion, the same haplotype, and an Amish–Mennonite ancestry, indicating a founder mutation. Furthermore, all five families had similar clinical features and a phenotype that was described as “mixed”. The term mixed was chosen because most but not all of the affected family members had clinical features that were intermediate yet distinct from those of early-onset, limb-predominant dystonia, which is associated with DYT1, and late-onset localised cervical and cranial dystonia, which constitutes the majority of primary dystonia. Similar to DYT1, the symptoms of DYT6 tend to begin early, and the dystonia usually spreads to involve multiple body regions. However, unlike DYT1, DYT6 is more likely to begin in cervical or cranial muscles. When DYT6 starts in the limbs it is much more likely to spread to cranial muscles, whereas disabling leg and gait abnormalities are less common in DYT6 than they are in DYT1. In addition to finding the Amish–Mennonite founder mutation in THAP1, we also discovered a unique missense mutation in one family that had clinical similarities to the Amish–Mennonite families. This family had European but not Amish–Mennonite ancestry, which suggests that THAP1 might also be a cause of dystonia outside the Amish–Mennonite population. We now report screening for DYT6 in DYT1-negative families that were not of Amish–Mennonite ancestry but have family members with early-onset non-focal primary dystonia, a phenotype that is consistent with DYT6.
Methods
Participants
Participating families were identified from a database that includes individuals recruited from the Movement Disorders Center at Beth Israel Medical Center, New York; Columbia-Presbyterian Medical Center, New York; Mount Sinai School of Medicine, New York; and through research advertisements. We identified 37 multiplex families in which at least one member had childhood-onset or adolescence-onset (i.e., before age 22 years) symptoms of dystonia with muscle involvement that was either segmental, multifocal, or generalised. Families without Amish–Mennonite ancestry were recruited, and we tried to assess all living family members who were affected by or had screened positive for dystonic symptoms. For all affected individuals, there were no clinical signs or laboratory findings of secondary dystonia, and mutation screening for the DYT1 GAG deletion was negative. One of the 37 families (family S) was also included in our initial report identifying mutations in THAP1; hence, 36 families were newly screened for mutations in THAP1. 104 affected family members in the 36 newly screened families had clinical information and DNA available for analyses. Family S and the four Amish–Mennonite families (M, C, R, and W) were described in the previous report, and are included here as a separate group to describe more fully the phenotype associated with mutations in THAP1 and for phenotype–genotype analysis.

Of the 104 affected family members, 86 had in-person or videotaped examinations and four had video examinations and available records. Each of the 14 remaining individuals was examined by a collaborating neurologist, and clinical information about age at onset, the muscles initially affected, and progression were obtained from medical records and telephone interviews. Interviews, reviews of medical records, and examinations were done in accordance with published protocols to ensure that the diagnosis and distribution of the affected sites were consistent with primary dystonia.11 Final clinical status was established through use of all available clinical information by two of the authors (SBB and RSP) who were blinded to a individual's DYT6 genotype. Age and site at onset were identified by self report and by review of medical records.

All participants gave informed consent before their participation in this study, which was approved by the Beth Israel Medical Center, Columbia-Presbyterian Medical Center, and Mount Sinai School of Medicine review boards.

Procedures
Blood samples were collected and DNA was extracted by standard methods. Screening for the three base pair deletion in the DYT1 locus was done as previously described.12 To investigate THAP1, we initially screened the index case from each of the 36 families. The three coding exons were fully sequenced, including the 5′ and 3′ untranslated regions (UTRs). To look for mutations at splice sites, at least 50 base pairs of the upstream and downstream intronic sequences that flank each exon were also sequenced. Standard polymerase chain reaction (PCR) amplification was done; details of the

![Figure 1: Partial pedigrees of affected individuals and relatives](image)
primers and conditions used are available on request. For exon one of 
\textit{THAP1}, AccuPrime™ GC-rich DNA polymerase (Invitrogen, Carlsbad, CA, USA) was used in the amplification. Standard dyeoxygen cycle sequencing was done on amplified fragments. In 277 randomly acquired human DNA control samples from healthy white blood donors in the UK (Sigma–Aldrich, St Louis, MO, USA), the 5′UTR and entire coding region of \textit{THAP1}, including the splice sites, was sequenced. The Amish–Mennonite haplotype was identified as previously described.¹

**Statistical analysis**

The clinical features of all affected individuals from families with a \textit{DYT6} mutation were compared with individuals from mutation-negative families. Because more than one member of a family was included, we used generalised estimating equation models for dichotomous variables and a random effects model in generalised least squares regression for continuous or ordinal variables, to control for the non-independence of individuals within the same family. These models were used to compare age at onset, site of onset, final distribution, sex, and final sites involved. Analyses were done with STATA, version 10 (Stata, TX, USA). We also assessed the phenotypic differences between individuals with deletions or nonsense mutations in \textit{THAP1}, which are predicted to produce truncated transcripts, and those with missense mutations.

**Role of the funding source**

The sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

**Results**

Of the 36 newly screened families, individuals in nine (25%) carried mutations; this group included 19 of 104 individuals (18%) affected with primary dystonia. Figure 1 shows the pedigrees and mutations in these families. Of note, a family member in family one and in family eight, who were rated to be affected, did not have the same mutation in \textit{THAP1} as the rest of the affected family members. Both of these individuals were men who had symptomatic brachial dystonia for 20 years but only when writing (writer’s cramp that began at age 6 years and 34 years, respectively). These individuals were deemed to be phenocopies and were not included in the analysis of either genetic group of families.

Eight of the nine families carried novel mutations (figures 1 and 2). Seven of these eight mutations were in the region that encodes the DNA-binding domain of \textit{THAP1} and included one nonsense mutation, five missense mutations (each of which resulted in the substitution of a residue that is highly conserved across species [figure 2]), and one small deletion that removed the start codon. The eighth mutation was a deletion of a

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**Figure 2: Mutations in \textit{THAP1}**

(A) Schematic of protein encoded by \textit{THAP1}: THAP domain (DNA binding; blue); low-complexity proline-rich region (red); coiled-coil domain (green); and nuclear-localisation signal (violet). The identified mutations are shown to scale, with the predicted substitution (missense) mutations on the top and the predicted truncating (nonsense and frameshift) mutations on the bottom. p?=deletion of T in the ATG codon of the first methionine of the protein and indicates that there is no experimental evidence on the expression of the protein product of this mutant. ^=previously identified mutations.¹ Mutation nomenclature taken from den Dunnen and Antonarakis,¹⁴ with numbering from the start codon.

(B) Multiple alignment of the \textit{THAP1} domain sequences from \textit{THAP1} orthologues. Protein sequences of the \textit{THAP}1 orthologues were retrieved from the NCBI gene database and aligned with ClustalW, version 2.0.9. The amino acid residues that are changed by the missense mutations and the first methionine codon are highlighted in blue. \textit{THAP}=thanatos-associated protein.
A single base pair that was predicted to result in a truncated protein; this would leave the DNA-binding domain intact but disrupt the nuclear-localisation signal. These mutations were not found in 554 chromosomes from white Europeans.

The ninth family had the same insertion/deletion mutation and haplotype as that identified in our Amish–Mennonite kindreds. This family was not aware of having an Amish–Mennonite background, but rather had mixed European ancestry. Ancestral countries of origin for the other eight families included: Ireland (n=3), Italy (n=2), Germany (n=2), and Russia (n=1). None reported Jewish ancestry. The 27 families in which mutations were not identified included 85 individuals with dystonia. Self-reported ancestry included mixed European (n=13), Ashkenazi Jewish (n=6), Irish (n=3), African (n=2), Italian (n=2), and French Canadian (n=1).

The table summarises the clinical features of individuals with identified mutations in THAP1. Among the newly described mutation-positive families, most individuals had childhood or adolescent onset, whereas three of 19 individuals had onset in adulthood (>21 years), at 25, 29, and 49 years, respectively. The first symptoms were often in an arm, although five of 15 individuals with arm onset reported simultaneous onset in other regions, including the other arm in three, a leg in one, and the other arm, neck, and tongue in another. Dystonia spread widely in most, with 12 of 19 affected individuals having generalised or multifocal distributions; even though one or both legs were involved in all, none required a wheelchair and most had only mild or moderate disability related to gait. Furthermore, all 12 affected individuals had cranial muscle involvement, which included the face, tongue, and larynx.
muscles were involved in 77% (37 of 48) and speech was affected in 67% (32 of 48). Although most of the affected individuals had widespread dystonia that affected the limbs, neck, and cranial muscles, five had more restricted disease: four had writer’s cramp and one had spasmodic dysphonia.

The oldest age at onset in the newly identified group was greater than that previously reported for DYT6; however, the median age at onset was not different between these groups (table). Clinical features were otherwise similar, except that the arm was more commonly the first site to be affected in the newly identified group.

AFFECTED INDIVIDUALS FROM FAMILIES WITHOUT IDENTIFIED MUTATIONS WERE SIGNIFICANTLY OLDER AT ONSET THAN THOSE IN THE MUTATION-POSITIVE GROUP (TABLE) DESPITE THE NARROW INCLUSION CRITERIA, WHICH REQUIRED AT LEAST ONE FAMILY MEMBER TO HAVE EARLY-ONSET NON-FOCAL DYSTONIA. THE DYSTONIA COMMONLY BEGAN IN THE CERVICAL MUSCLES, UNLIKE FOR THOSE WITH MUTATIONS IN THAP1 (60% [51 of 85] vs 17% [8 of 48]), WHEREAS THE ARM WAS FIRST AFFECTED IN ONLY 19% (16 of 83) OF MUTATION-NEGATIVE INDIVIDUALS COMPARED WITH 58% (28 of 48) OF THOSE WITH MUTATIONS. SIMILARLY, THERE WAS A DIFFERENCE IN THE FREQUENCY OF ARM INVOLVEMENT (51% [43 of 85] vs 92% [44 of 48]). COMPARED WITH THE MUTATION-POSITIVE GROUP, SPEECH WAS LESS FREQUENTLY AFFECTED, THERE WAS LESS EXTENSIVE MUSCLE INVOLVEMENT, AND A SMALLER PROPORTION PROGRESSED TO A GENERALISED DISTRIBUTION (22% [19 of 85] vs 46% [22 of 48]). CONVERSELY, THE DYSTONIA REMAINED FOCAL IN A HIGHER PROPORTION OF MUTATION-NEGATIVE INDIVIDUALS (36% [31 of 85]), WITH THE NECK AS THE MOST COMMON SITE (61% [19 of 31]). DESPITE THESE DIFFERENCES, SEVERAL MUTATION-NEGATIVE FAMILIES HAD CLINICAL FEATURES THAT WERE SIMILAR TO THOSE IN MUTATION-POSITIVE FAMILIES, INCLUDING AN EARLY ONSET WITH SPREAD TO MULTIPLE REGIONS AND PROMINENT CRANIAL INVOLVEMENT THAT AFFECTED SPEECH.

To assess the relation between genotype and clinical expression, we divided the 14 families that carried THAP1 mutations into two groups: those with predicted truncating mutations (eight families, including the original Amish–Mennonite kindred) and those with missense mutations (six families). We found no differences in age at onset, sites of onset, or final distribution (data not shown).

**Discussion**

Mutations in THAP1, which cause DYT6 dystonia, are associated with a substantial proportion of familial early-onset primary dystonia; 25% of our 36 families had mutations in THAP1. Of the nine families with mutations, one family had the five base pair insertion/three base pair deletion and haplotype of the Amish–Mennonite kindred in whom DYT6 was first mapped. The remaining eight families each had unique mutations in one of the three exons of THAP1 and were also from a diverse range of ancestries, although all were of European non-Jewish origin. There were no phenotypic differences that corresponded to mutation type; however, the identified mutations either affected the DNA-binding domain or removed the nuclear-localisation signal of THAP1, which suggests that these domains are functionally important (figure 2). The DNA-binding domain is essential for the pro-apoptotic role that is associated with this protein: THAP1 is a nuclear pro-apoptotic factor that interacts with prostate-apoptosis-response-4 (Par-4) and colocalises with it to promyelocytic leukaemia nuclear bodies.

We have proposed a role for mutated THAP1 in populations other than the Amish–Mennonite population, on the basis of one German family that had a different missense mutation. This family, similar to the Amish–Mennonite kindred, has a mixed phenotype; therefore, the first step was to screen families from other populations with a consistent phenotype (ie, those with at least one member with early-onset and non-focal dystonia). Despite our clinical restrictions in defining the group of families for screening, we identified differences between those with and those without mutations: mutation-positive individuals had an earlier-onset dystonia that had a greater tendency to generalise and more commonly involved brachial, facial, and oromandibular muscles than did the mutation-negative individuals. These differences are consistent with the previously reported characteristics of the mixed phenotype of DYT6.

Because we specifically excluded individuals with the DYT1 GAG deletion, we did not fully depict the range of early-onset dystonia and the distinct clinical features associated with THAP1 in this population compared with DYT1. For example, although many individuals with mutations in THAP1 had onset in childhood, and 90% began before age 30 years, more than 30% had onset after 18 years, which is a higher proportion than that reported for DYT1. Also, compared with DYT1, leg onset was less common in DYT6. Perhaps the most distinctive characteristic of DYT6, which distinguishes it from DYT1, was the proportion of affected individuals with involvement of the cranial muscles, either at onset (29%) or later (77%). In most of these individuals, this produced disabling dysarthria or dysphonia. Finally, unlike DYT1, more women than men were affected. The higher proportion of affected women in families with DYT6 has been noted previously but remains unexplained. The lifetime penetrance of DYT6 is estimated as 57–60%, and no sex differences have been identified; however, few male gene carriers were studied. The families reported here also included unaffected mutation carriers, which is consistent with reduced penetrance (figure 1). Future systematic assessment of families with DYT6 should help to resolve whether a sex difference in penetrance can be shown.

Although we were able to confirm an overall distinct clinical phenotype for DYT6, there are several caveats to our study. One important caveat is the recognition that the clinical expression of DYT6 is broad and overlaps with non-DYT6 dystonia subtypes. This applies to other subgroups of dystonia that have prominent speech involvement (eg, DYT4, DYT17, and DYT12) and the
common phenotype of brachial and bi-brachial dystonia. Indeed, we identified two family members in mutation-positive families who had writer’s cramp but did not carry the family mutation. Writer’s cramp is a particularly problematic phenotype for genetic studies10,11 and the need for caution when classifying individuals as “affected” for gene mapping is reinforced by our study. Also, mutation analysis was restricted to sequence variants within the regions that were screened. Screening for other genetic changes, including exon deletions, multiplications, and variations in the intronic regions, might identify additional affected individuals and requires further study. Finally, we only assessed a population that is consistent with the initially described DYT6 phenotype; other dystonia populations, particularly sporadic and late-onset focal dystonias, were untested.

Future studies should help to clarify the role of mutations in THAP1 in dystonia and their pathogenic effects. On the basis of the mutations identified, the DNA-binding and pro-apoptotic functions of THAP1 are implicated; transcription factors and apoptosis have not been associated with primary dystonia, although TAF1, the gene that causes DYT1, yet metabolic differences between DYT1 and DYT6 genotypes were also shown. This suggests that mutant torsinA and THAP1 have distinct pathogenic origins and might converge on key brain targets to produce a common motor phenotype. Understanding the function of THAP1 and investigating its potential interactions with torsinA should provide new mechanistic clues and targets for therapy.

Contributors
SB is the principal investigator for the study; she wrote the manuscript and reviewed patient videotapes to confirm they were affected. DR coordinated and participated in the collection of much of the data and worked on the statistical analysis. TF and LO did the molecular analysis. RS-P examined the individuals and reviewed videotapes of those she did not examine; she also supervised the statistical analyses of the study data. GH contributed to the content and the revision of the manuscript, the table, and the figures.

Conflicts of interest
We have no conflicts of interest.

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