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Heterogeneity in primary dystonia: lessons from *THAP1, GNAL* and *TOR1A* in Amish-Mennonites

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Abstract

Background—A founder mutation in the *THAP1* gene causing primary dystonia was originally described in the Amish-Mennonites. However there may be both genotypic and phenotypic heterogeneity of dystonia in this population that may also inform studies in other ethnic groups.

Methods—Genotyping for THAP1, and GNAL mutations and genotype-phenotype comparisons were performed for 76 individuals of Amish-Mennonites heritage with primary dystonia.

Results—27 had mutations in *THAP1*—most with the founder indel mutation, but two had different *THAP1* mutations; 8 had mutations in *GNAL;* and 1 had a *de novo* GAG deletion in

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TOR1A. In the primary analysis comparing *THAP1* carriers to all non-*THAP1*, *non-GNAL*, *non-TOR1A* individuals, age at onset was lower in *THAP1* carriers (15.46±9.20, range 5–38 vs. 39.18±17.65, range 1–70, p<0.001), carriers were more likely to have onset of dystonia in an arm (44.4% vs. 15%, p=0.02), and to have arm (88.9% vs. 22.5%, p<0.01), leg (51.9% vs. 10% p=0.01), and jaw/tongue (33.3% vs. 7.5%, p=0.02) involvement at final examination. Carriers were less likely to have dystonia restricted to a single site (11.11% in carriers vs. 65.85% (p<0.01)), and less likely to have dystonia onset in cervical regions (25.9% *THAP1* vs. 52.5% in non-carriers, p=0.04).

Conclusions—Primary dystonia in the Amish-Mennonites is genetically diverse, and includes not only the *THAP1* indel founder mutation but also different mutations in *THAP1* and *GNAL* as well as the *TOR1A* GAG deletion. Phenotype, particularly age at onset combined with final distribution, may be highly specific for the genetic etiology.

Keywords

genetics; dystonia; *THAP1*; *GNAL*; Amish; Mennonites

Introduction

Dystonia due to mutations in *THAP1* (DYT6) is characterized by early onset primary dystonia with prominent cranio-cervical, and frequent arm involvement.[1] DYT6 linkage was initially established in two large Amish-Mennonite families,[2] and the linked region was subsequently narrowed using two additional Amish-Mennonite families with a similar phenotype.[3] We then determined that dystonia in these families, as well as in apparently non-Amish-Mennonite individuals, was due to an insertion/deletion (indel) mutation in exon 1 in the Thanatos-associated [THAP] domain-containing apoptosis-associated protein 1 gene (*THAP1*), which caused the insertion of a premature stop codon.[4] To date, over 80 mutations have been reported as causative for *DYT6* in a wide range of ethnic groups including European (English, Dutch, German, French, Serbian, Swedish, Italian, Greek), Iranian, Indian and Chinese populations.[1, 5–13]

Although the genetic etiology of *DYT6* was first determined in Amish-Mennonites, not all Amish-Mennonites affected with PTD share the *THAP1* founder mutation[3] suggesting genetic heterogeneity in this population. Indeed, a previously reported case of an Amish-Mennonite man with primary torsion dystonia (PTD) with a *de novo TOR1A* mutation[14] and two Amish-Mennonite families with primary dystonia who did not show linkage to the DYT6 region[3] indicate that other genes are responsible for primary dystonia in this population. The elucidation of *THAP1* and more recent identification of *GNAL* as a causative gene for dystonia prompted us to study the role of the three primary dystonia genes, *TOR1A, THAP1* and *GNAL* in the Amish-Mennonites. We also sought to determine whether apparent phenocopies in the Amish-Mennonites are attributable to other *THAP1 or to GNAL* mutations.

Methods

Participants

As part of a larger genetic study to identify a gene for dystonia in Amish-Mennonite individuals, we evaluated a total of 76 affected individuals from 40 families with at least one grandparent of Swiss-German Amish or Mennonite descent who settled in Pennsylvania, Ontario, Ohio, Indiana or Illinois. Individuals were referred by physicians and by the Dystonia Medical Research Foundation, or answered advertisements in an Amish newspaper. Because families were expanded to identify as many affected individuals as possible, there is a bias toward familial cases. While many of the Amish-Mennonites solely identify as Mennonite, in some families there are Amish ancestors; therefore, we have denoted the group overall as Amish-Mennonite.[15] We excluded those individuals who descended exclusively from Dutch-Russian (Dutch-German) Mennonite ancestors, as this group is genetically distinct from the Amish-Mennonite (Swiss-German) group.[16] One family having one grandparent of Dutch-Russian Mennonite background and one of Amish-Mennonite background was retained. All subjects gave informed consent prior to participation in this study, which was approved by the institutional review boards at Beth Israel Medical Center and Mount Sinai School of Medicine. The individuals were interviewed and examined according to previously published protocols,[17] including videotaped examinations. Blood for DNA extraction was obtained. When possible, first- and second-degree family members were also examined, and blood samples were collected. Affected individuals reporting clinical signs or laboratory findings suggestive of secondary dystonia were excluded. Three authors (SBB, MSL, RSP) blinded to genotype results, reviewed standardized videotaped examinations. Definite dystonia was defined as characteristic overt twisting or directional movements and postures that were consistently present. Ratings for probable, possible and no dystonia were defined as previously reported.[18] When in-person or videotaped examination was not possible, but medical records confirmed the diagnosis of dystonia, individuals were categorized as definite by history.

Final clinical status was determined by two authors (SBB and RSP), after considering the evaluations of on-site examiners, video review examiners, and any additional information available from medical records. Age and site at onset were determined by self-report and review of medical records. All clinical evaluations and the final decisions of affected status were made prior to ascertaining genotype.

Four Amish-Mennonite families were previously reported: three were clinically described (M, C, and R)[2, 3] and reported as carrying the *THAP1* indel founder mutation,[4] and the fourth was included in our report describing additional *THAP1* mutations.[1] Two *GNAL* families were described briefly in the initial publication describing *GNAL* mutations as causative for dystonia.[19]

In total, there were 76 affected individuals with clinical information and DNA available for analyses. Of the 76, 62 had in-person and videotaped exams, while 2 had video examinations and medical records available. The remaining 12 individuals were evaluated by neurologists specializing in movement disorders, and information was derived from

medical records and telephone interviews. If there were multiple examinations, information from the final examination is reported. The Beth Israel IRB approved this study.

Molecular Analysis

Blood samples were collected from participants and DNA was extracted using standard methods. Screening for the three base pair deletion in *TOR1A* was performed as previously described.[20] For *THAP1*, we initially screened the index case from each of the 40 families by direct Sanger sequencing as previously described.[4] In families with the *THAP1* indel mutation, haplotypes were constructed as previously reported.[4] *GNAL* mutations were assessed as described.[19]

Statistical Analysis

Clinical features of all affected individuals from families with *THAP1* mutations were first compared to all individuals without *THAP1*, *GNAL* or *TOR1A* mutations. Features included: gender, age at onset, site onset, final distribution, and final sites involved. As we included more than one member of a family, mixed effects logistic regression models were applied to account for the correlations among measurements of subjects from the same family. The analysis was then repeated comparing *THAP1* to *GNAL* mutation carriers. Analyses describing sensitivity and specificity of prescribed cut-points of age of onset, and sites previously described as associated with *THAP1* mutations were also performed.[1] Analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC).

Results

Among the over 300 Amish–Mennonites evaluated as part of our Genetics of Primary Dystonia study since 1992, we report 76 with definite primary dystonia, among whom 27 had *THAP1* mutations, eight had *GNAL* mutations, and one a *TOR1A* mutation. Of these 36 mutation positive affecteds (from 16 families) 31 were previously reported (1 *TOR1A*, 23 *THAP1*, and 7 *GNAL*). We identified two new unique *THAP1* mutations, c.65T>C; p.F22S and c.67C>T; p.H23Y, as well as two new individuals with the insertion/deletion (indel) mutation c.135_139delinsGGGTTTA; p.F45fs73X, who were not known to be closely related to the originally described families (Table 1). All indel mutation individuals shared the common haplotype.[4] The two new missense mutations were conserved across vertebrate *THAP1* orthologs and were not found in dbSNP137, ~3,500 European exomes in the NHLBI Exome Sequencing Project database or 572 control chromosomes that we tested.

The individual with the c.65T>C; p.F22S mutation (Family A) had onset at age 12 with cervical dystonia, which then spread to involve upper and lower face, including jaw and tongue, both arms, legs and trunk. Leg dystonia was mild and did not significantly impair her walking. Cervical dystonia improved with botulinum toxin A injections, and there was only partial response to anticholinergic medications. In addition, she had a distant cousin with a *de novo TOR1A* mutation and classic early onset leg dystonia significantly affecting gait, but without cranial involvement,[14, 21] and a first cousin once-removed with blepharospasm beginning at age 60 with neither a *THAP1* nor *TOR1A* mutation (Figure 1, Pedigree). The second novel mutation carrier (c.67C>T; p.H23Y) developed right arm

dystonia by age 7, which spread to prominently involve her neck and eventually both legs. The remaining 40 subjects did not harbor *THAP1* mutations and included two families with predominant cervical dystonia. In addition to the two previously described *GNAL* mutations,[19] we identified a novel *GNAL* mutation, c.514G>A; p.V172I, in a woman with onset of laryngeal dystonia at age 21, that progressed to involve cervical muscles. This mutation was not seen in dbSNP137, ~3,500 European exomes in the NHLBI Exome Sequencing Project database or 572 control chromosomes that we tested. The V172 amino acid is also conserved among *GNAL* orthologs. There was no family history of dystonia, but there was a strong history of tremor. The patient's father had voice and head tremor that began in his 50s, and her son and brother had arm tremor. As none of the family members were available for examination, we cannot comment on whether this tremor in the relatives was dystonic. However, the mutation was present in the son with arm tremor, the only other family member with a DNA sample. The three *GNAL* mutations were private in each family and no other *GNAL* mutations were identified in this population.

The clinical features of subjects with identified *THAP1* mutations are summarized in Table 2. The *THAP1* mutation positive group was younger at examination than the mutation negative group (15.5±9.2 years vs. 39.2±17.7, $p<0.001$) and the *GNAL* mutation group (50.3±10.4, $p=0.07$). Duration of dystonia was not different between groups: THAP1, 21.0±9.2 years; no mutation, 14.0±10.9; and GNAL, 21.3±13.3. Women were overrepresented in *THAP1* and mutation negative groups (70.4% of *THAP1* mutation carriers, and 67.5% in non-carriers), but not GNAL (37.5%).

The phenotype of the *THAP1* mutation positive individuals was characterized by dystonia onset in an arm for many (44.44%), followed by neck (25.93%) and craniofacial muscles (25.93%). A larger proportion of carriers had arm onset than those without mutations (44.4% vs. 15.0%, $p=0.02$), and mutation negative individuals were more likely to have cervical onset (52.5% vs. 25.9%, $p=0.04$). Only one individual had onset of dystonia in a leg and an arm, and another in both legs. Age at onset was lower in the *THAP1* mutation positive group than the mutation negative group (15.5±9.2, range 5–38 vs. 39.2±17.7, range 1–70, $p<0.0001$). Compared with non-carriers, *THAP1* mutation carriers were more likely to have arm (88.9% vs. 22.5%, $p<0.001$), leg (51.85% vs. 10.0%, $p=0.01$), and jaw or tongue (33.3 vs. 7.5, $p=0.02$) involvement at final examination. They were also more likely to have dystonia spread to another site (88.9% vs. 34.88%, $p=0.02$).

When compared with the *GNAL* mutation group, *THAP1* carriers were more likely to have onset in an arm (44.4% vs. 0.0%, $p=0.023$). Leg onset was infrequently present in both groups (12.5% of *GNAL* carriers and 7.4% of *THAP1* carriers, $p=0.66$). Compared with *GNAL* carriers, *THAP1* mutation carriers were more likely to have arm involvement at final examination (88.9% vs. 37.5%, $p<0.02$). Age at onset was lower in the *THAP1* group than the *GNAL* group (15.5±9.2 vs. 32.9±10.7, range 20–48, $p<0.0001$). While there was a greater proportion of women and girls affected in the *THAP1* group, this was not significantly different.

THAP1 mutation carriers represented 62.5% of the Amish-Mennonite group with onset at age 21 or younger, 54.8% of the group with onset less than age 30, and none were

represented in the group with onset at age 60 or older. *TOR1A* was rare in this group, with a mutation in only one of 32 (3.1%) early-onset cases (onset ≤21 years). Among subjects with onset ≤21, sensitivity for arm AND speech involvement at final examination was low for *THAP1* mutation (7/21= 33%, 95% confidence interval (CI) 0.17–0.55), but specificity was high (7/9 =85%, CI 0.58–0.96). Specificity was slightly higher than final site involving speech alone in the ≤21 year onset group (77%, CI 0.50–0.90, with sensitivity 38%, CI 0.21–0.59), and sensitivity was greatest for final site involving an arm in the early age of onset subgroup (86%, CI 0.65–0.95 and specificity 46%, CI 0.23–0.70).

Discussion

We demonstrate that in addition to the common *THAP1* founder mutation, at least two other *THAP1* mutations, multiple independent *GNAL* mutations and a *de novo TOR1A* GAG deletion cause primary dystonia in Amish-Mennonites. Because *DYT6* was mapped in related Amish-Mennonite families and a founder haplotype and mutation in these families allowed for gene identification, the founder *THAP1* mutation is commonly equated with all primary torsion dystonia in this population. However, the findings of additional *THAP1* mutations, as well as *TOR1A* and *GNAL* mutations and mutation-negative individuals of Amish-Mennonite descent, emphasizes the genetic heterogeneity of primary dystonia, even in this ethnic isolate. Our results also underscore that phenotype remains a major predictor in determining genotype. This has both counseling and treatment implications.

Although genotype specific therapies do not yet exist, knowing genotype may affect decisions regarding current symptomatic treatment. Specifically deep brain stimulation (DBS) response may vary by genetic etiology, with DYT1 mutation carriers demonstrating a better response than DYT6. Individuals with *TOR1A* mutations show more consistent improvement overall with bilateral globus pallidum (GPi) DBS than non-DYT1 dystonia cases,[22] and *THAP1* cases in particular,[3] where there is usually incomplete resolution of dystonia.[24] However, the number of THAP1 cases reported is small compared to DYT1, and response to GPi DBS among *THAP1* patients varies among series[23]. If the potentially less robust response to DBS for *THAP1* is maintained in larger studies, this would have implications both for prognosticating surgical benefit with GPi stimulation and considering other targets in DYT6 patients. The only patient in our cohort who underwent DBS, and for whom records are available was the *DYT1* carrier, who demonstrated excellent response bilateral GPi DBS surgery at age 42.

The typical *THAP1* dystonia phenotype is characterized by predominantly early disease onset with brachial and speech involvement, that often generalizes but with non-disabling mild leg dystonia.[1] Two studies concluded that mutations within the THAP DNA binding domain tend to manifest at an earlier age and exhibit more extensive anatomical distributions than mutations localized to other regions of *THAP1*.[25, 26] We could not assess that correlation as all of the mutations we identified occurred in the DNA binding domain. However, within the families carrying the F45fs73X founder mutation, there is a wide range of phenotypes suggesting that other factors beyond mutation location influence phenotypic expression.

Although our study was not well powered to compare *GNAL* mutation carriers as a group to others, it is of interest that none of the seven *GNAL* carriers had the classic *THAP1* phenotype of early onset (≤21 years) with both arm and speech affected. Further, even among this small group, a relatively older age of onset was detected in comparison with *THAP1* mutation carriers. Overall, the phenotypes defined in the Mennonite population for *THAP1* and *GNAL* mutation carriers, are consistent with what has been reported for other primary dystonia cohorts.[5, 11, 19, 24–35]

It is also important to note that there are Amish and Amish Mennonite founder mutations for several non-primary forms of dystonia including pantothenate kinase associated neurodegeneration (PKAN), glutaric aciduria type 1, and homocystinuria due to a founder mutation in methyltetrahydrofolate reductase (reviewed in[15]). Further, in our recruitment for genetic studies, we evaluated an 8-year-old Amish girl with prominent dystonia, but also ocular telangiectasias, oculomotor apraxia, chorea and ataxia, who was discovered to have one of the two Amish founder mutations in the *ATM* gene. While ataxia-telangiectasia in its classic and most common form presents with prominent ataxia and telangiectasia, its variant form may present as primary dystonia without cerebellar atrophy, ataxia or telangiectasia.[36] Hence the diagnostic net, even in apparently primary dystonia in Amish-Mennonites, should be broad.

Although we did not screen for the three recently identified primary dystonia genes, ANO3,[37] TUBB4a,[38, 39] and CIZ1,[40] it is possible that these may be present in the mutation negative group. However, if mutations in these genes were identified in a small subset of non-mutation cases, this would only further support our findings of genetic heterogeneity in this population.

The genetic heterogeneity and relationship between phenotype and genotype is highlighted in Family A: the Amish-Mennonite proband with a classic DYT6 phenotype with early onset dystonia involving both arm and cranial sites did not harbor the founder indel, but another *THAP1* mutation; her aunt with definite focal blepharospasm did not have any mutation in the three genes tested; and her distant cousin with early onset dystonia not involving cranial muscles/speech but which prominently affected the leg limiting gait, had a *de novo* DYT1 mutation. For all three, the phenotype is helpful in determining genotype. Our analyses of all subjects further support using clinical features particularly age of onset and distribution, to guide testing decisions; we found excellent specificity but moderate sensitivity and the confidence intervals were broad. Larger samples, not biased for familial cases, are needed to further determine testing characteristics for *GNAL* and *THAP1* dystonia.

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Dr. Bressman serves on the advisory boards of the Michael J. Fox Foundation, the Dystonia Medical Research Foundation, the Bachmann Strauss Dystonia and Parkinson's Foundation, and the Board of We Move. She has consulted for Bristol Meyer Squibb. She has received research support from the Michael J. Fox Foundation, National Institutes of Health (NIH), and Dystonia Medical Research Foundation. Dr. Bressman received royalty payments from Beth Israel/Mount Sinai/Athena for DYT6 testing.

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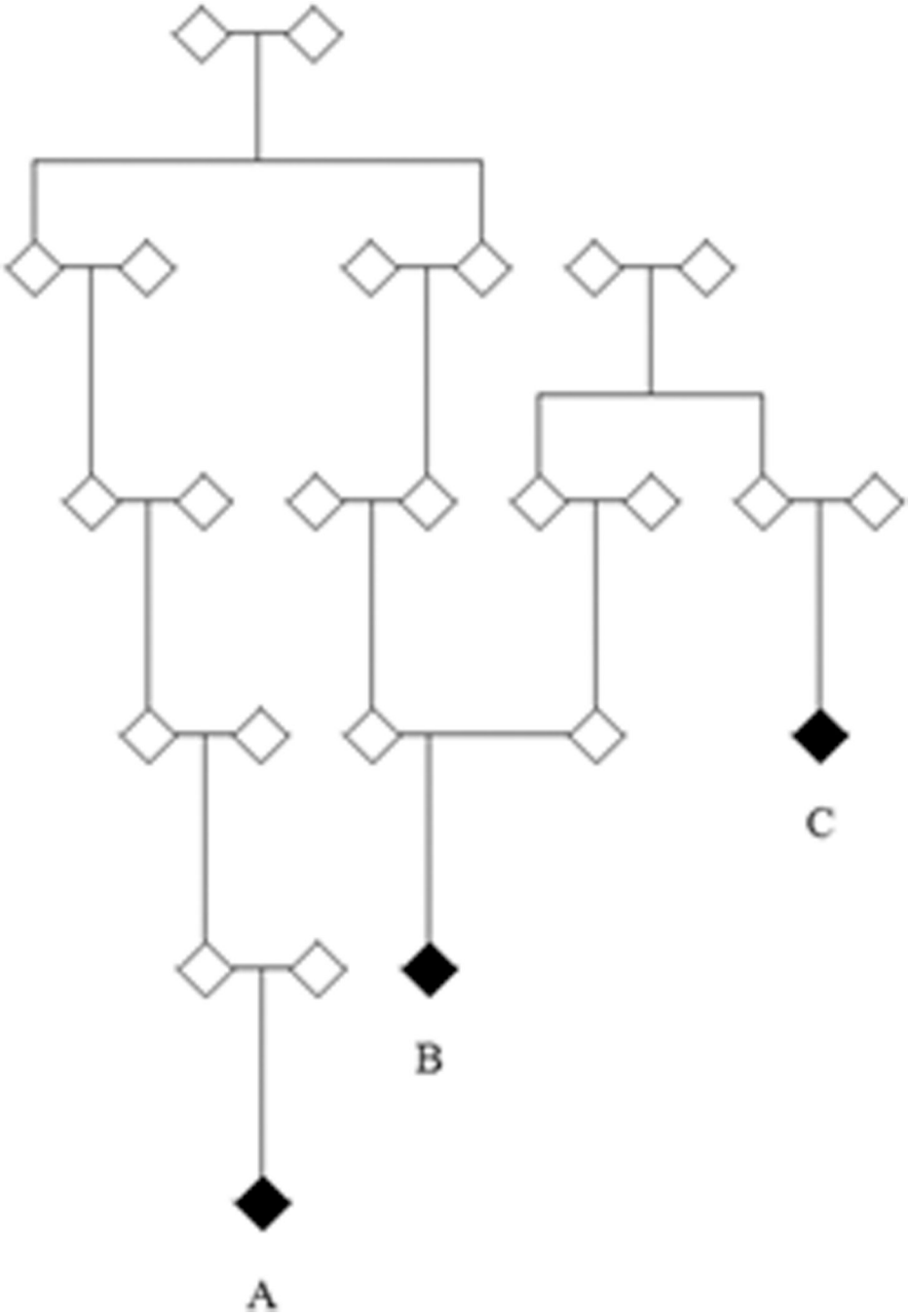

Figure 1. Pedigree demonstrating three different genetic etiologies of dystonia in the described extended Amish-Mennonite family

A has childhood onset DYT1 dystonia with prominent leg involvement and no voice. **B** has childhood onset DYT6 dystonia with prominent cranio-cervical as well as arm and trunk dystonia that is due to a *THAP1* mutation not the founder. **C** has adult onset blepharospasm without a known mutation.

Figure 2. Age at onset curves comparing distributions by mutation

Table 1

Clinical features in the newly described *THAP1* mutation and *GNAL* mutation carriers

Person	Mutation *THAP1*	Sex	Age Onset	Age Exam	Site Onset	Sites ever involved	Distribution	Family history dystonia
1	c.67C>T p.H23Y	F	7	50	Arm	ANRG	generalized	no
2	c.65T->C p.F22S	F	12	31	Neck	FTJNAMKRG	generalized	yes
3	135_139delinsGGGTTTAp. F45fs73X	F	18	18	Neck	NAKMRG	generalized	no
4	135_139delinsGGGTTTAp. F45fs73X	F	10	53	Arm	ANF	segmental	yes
	GNAL							
5	c.514G>A p.V172I	F	21	60	Larynx	LN	segmental	no

F=upper face, T=tongue, J=jaw, L=larynx, N=neck, A=right arm, M=left arm, K=trunk, R=right leg, L=left leg

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Table 2

Clinical features comparing *THAP1* mutation group to no-mutation group and *GNAL* group

	***THAP1* Mutation positive n=27, families=7**	**Mutation negative (no *THAP1*, no *GNAL*, no DYT1)*** n=40, families=30**	**P-value *THAP1* vs. no mutation**	***GNAL* mutation n=8,families=3**	**P-value *THAP1* vs. *GNAL***
Women	19 (70.4%)	27 (67.5%)	0.8	3 (37.5%)	0.09
Mean age onset (years ± SD, range)*	15.46±9.2 (5–38)	39.18±17.65 (1–70)	<0.001	32.9 ± 10.7 (20–48)	<0.001
Mean age at exam (years ± SD, range)	37.41 ± 18.78 (10–78)	53.18 ± 15.24 (20–82)	<0.001	50.25 ± 10.24 (38–68)	0.07
Mean duration of dystonia,(years ± SD, range)	21 ± 17.28 (1–64)	14 ± 10.94 (1–53)	0.18	21.25 ± 13.25 (3–39)	0.66
Site of onset					
Arm	12 (44.44%)	6(15%)	0.02	0 (0%)	0.02
Leg	2 (7.41%)	2 (5%)	0.69	1 (12.5%)	0.66
Cranial	7 (25.93%)	12 (30%)	0.72	1 (12.5%)	0.45
Face	2 (7.41%)	7 (17.5%)	0.26	0 (0%)	0.44
Jaw/tongue	2 (7.41%)	1 (2.5%)	0.57	0 (0%)	0.5
Larynx	3 (11.11%)	4 (10%)	0.92	1 (12.5%)	0.51
Cervical	7 (25.93%)	21 (52.5%)	0.04	6 (75%)	0.12
Sites at examination					
Arm	24 (88.89%)	9(22.5%)	<0.001	3 (37.5%)	0.02
Leg	14 (51.85%)	4 (10%)	0.01	2 (25%)	0.27
Cranial	18 (66.67%)	20 (50%)	0.22	3 (37.5%)	0.16
Face	13 (48.15%)	15 (37.5%)	0.33	2 (25%)	0.23
Jaw/tongue	9 (33.33%)	3 (7.5%)	0.02	0 (0%)	0.09
Larynx	9 (33.33%)	8 (20%)	0.6	1 (12.50%)	0.74
Cervical	18 (66.67%)	25 (62.5%)	0.41	8 (100%)	0.18
Distribution					
Focal	3 (11.11%)	27 (67.5%)	<0.001**	3 (37.50%)	0.10**

	***THAP1* Mutation positive n=27, families=7**	**Mutation negative (no *THAP1*, no *GNAL*, no DYT1)*** n=40, families=30**	**P-value *THAP1* vs. no mutation**	***GNAL* mutation n=8,families=3**	**P-value *THAP1* vs. *GNAL***
Segmental	10 (37.04%)	9 (22.5%)		3 (37.50%)	
Multifocal	4 (14.81%)	2 (5%)		0 (0%)	
Generalized	10 (37.04%)	2 (5%)		2 (25%)	

* Age of onset missing for one DYT6 carrier

** Group comparisons between focal vs. non-focal distribution

*** DYT1 GAG deletion carrier is not included, therefore only 75 individuals described in the table