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The c.-237_236GA>TT *THAP1* Sequence Variant Does Not Increase Risk for Primary Dystonia

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Abstract

Sequence variants in coding and non-coding regions of *THAP1* have been associated with primary dystonia. In this study, 1446 Caucasian subjects with mainly adult-onset primary dystonia and 1520 controls were genotyped for a variant located in the 5'-untranslated region of *THAP1* (c.-237_236GA>TT). Minor allele frequencies were 62/2892 (2.14%) and 55/3040 (1.81%) in subjects with dystonia and controls, respectively ($P = 0.202$). Subgroup analyses by gender and anatomical distribution also failed to attain statistical significance. In addition, there was no effect of the TT variant on expression levels of *THAP1* transcript or protein. Our findings indicate that

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the c.-237_236GA>TT *THAP1* sequence variant does not increase risk for adult-onset primary dystonia in Caucasians.

Keywords

dystonia; DYT6; high-resolution melting; untranslated region; THAP1

DYT6 dystonia is an autosomal dominant primary dystonia causally-associated with sequence variants in *THAP1* which encodes the DNA-binding transcription factor THAP1.1⁻3 DYT6 dystonia shows reduced penetrance and variable expressivity.2⁻8 In contrast to DYT1 dystonia, DYT6 more commonly remains focal in distribution and often affects the cervical and laryngeal musculature.1,4,5 Over 30 sequence variants have been localized to the coding regions of *THAP1* and associated with focal, segmental, multi-focal or generalized dystonia with age of onset ranging from 2 to 62 years.1⁻8 In addition, several asymptomatic carriers have been identified in the relatives of probands. The genetic and phenotypic heterogeneity of DYT6 dystonia, and variable penetrance of known coding variants suggest that non-coding variants in *THAP1* could contribute to the risk of developing adult-onset primary dystonia.4,5,7 Given that THAP1 is a transcriptional repressor, it is conceivable that non-coding variants which cause minor quantitative changes in the temporal or spatial patterns of THAP1 expression could have broad effects on the transcriptome.

Djarmati and colleagues4 identified a non-coding sequence variant (c.-237_236GA>TT) near the transcriptional start site of *THAP1* that might increase the risk of developing primary dystonia. The relative frequency of this polymorphism in their subjects with dystonia (20/320) relative to controls (7/355) was noteworthy ($P = 0.0054$). Extrapolation of their findings to a broad population of late-onset primary dystonia is problematic given that the subjects with dystonia were relatively young (mean age of onset = 38.5 years) and predominantly Northern German whereas the control group was composed of Caucasian individuals of more diverse European ancestry. Another, relatively small case-control study with heterogeneous control and dystonia populations did not find an association between the TT allele and risk for dystonia.7

Herein, we present the results of a large case-control study of c.-237_236GA>TT in primary, mainly adult-onset, dystonia. All subjects were Caucasians. Moreover, the effects of the TT variant on overall gene expression (transcript) were interrogated in leukocytes, and replicated in lymphoblastoid cell lines (transcript and protein) derived from distinct cohorts of cases and controls. Although RNA derived from peripheral blood has been used to study DYT1 dystonia9 and other movement disorders, analysis of gene expression in lymphoblastoid cell lines limits potential exogenous confounds including the effects of medications, nutrient intake, and concomitant infectious and inflammatory medical conditions.

PATIENTS AND METHODS

Participants

All human studies were conducted in accordance with the Declaration of Helsinki with formal approval from the institutional review boards at each participating study site. All subjects gave written informed consent. Recruitment of patients with primary dystonia and neurologically-normal controls is described in Xiao et al.5 Additional Caucasian control samples were obtained from Sigma-Aldrich (Human Random Control DNA Panels 1, 3, and 4), Emory Center for Neurodegenerative Disease Tissue Bank Core, Washington University

in St. Louis School of Medicine Neuroscience Blueprint Core and Coriell Institute for Medical Research (Control Panels NDPT020 and NDPT024). All normal controls except those from Sigma-Aldrich were examined to exclude dystonia and other neurological disorders. Demographics for dystonia and control subjects are presented in Table 1. Of note, Table 1 does not include family members of probands.

High Resolution Melting

High resolution melting (HRM) analyses were performed with the LightCycler® 480 Real-Time PCR system and High Resolution Master Mix (Roche) in accordance with manufacturer instructions and our laboratory protocol5 using forward (acctggcctcagccaatagt) and reverse (ctgcgctcggttggattc) primers designed to amplify the 5'-untranslated region (UTR) of *THAP1*. Melting curves and difference plots were analyzed by three investigators (J.X., Y.Z. and M.S.L.) blinded to phenotype. All samples were unambiguously assigned to genotypes by Gene Scanning software. For samples with shifted melting curves, PCR products were cleaned using ExoSAP-IT® (United States Biochemical) and sequenced in the forward and reverse directions. To evaluate the sensitivity and specificity of HRM, amplicons from 400 neurologically-normal controls and 400 subjects with dystonia were subjected to Sanger sequencing. Fisher's exact test was used to evaluate association of the c.-237_236GA>TT sequence variant with dystonia. The Genetic Power Calculator, case-control discrete traits module, was used for power analysis.10

THAP1 Expression

Ambion's LeukoLOCK™ Total RNA Isolation System and TRI Reagent® were used to isolate RNA from peripheral blood leukocytes of dystonia subjects and controls. Leukocyte RNA was used to evaluate the effects of the TT allele on THAP1 expression in dystonia subjects with the TT allele ($n = 20$) and controls without the TT allele ($n = 24$). RNA and protein were also extracted from lymphoblastoid cells derived from another 5 patients with the TT allele and 10 normal controls without the TT allele. Sanger sequencing was used to exclude coding, splice-site, 5'UTR and previously reported intronic sequence variants (c.71+9C>A, c.71+126T>C) from the control and dystonia groups.5,7 Detailed protocols for maintenance of lymphoblastoid cell lines, quantitative real-time PCR and quantitative Western blotting are provided in Supporting Information Methods. Student's *t*-tests were used to compare RNA and protein expression between dystonia and control samples. G*Power 3 was used for post-hoc power analysis.11

RESULTS

As depicted in Supporting Information Figure 1, melting curves robustly discriminated GA/GA homozygotes from heterozygotes (GA/TT) and homozygotes of the minor allele (TT/TT). Furthermore, difference plots clustered these genotypes into three discrete groups. Based on follow-up sequencing of samples exhibiting shifted melting curves and sequencing data from 400 neurologically-normal controls and 400 subjects with dystonia, HRM showed 100% diagnostic specificity and sensitivity.

As detailed in Table 1, only one subject with dystonia was homozygous for the TT allele whereas 60 were GA/TT heterozygotes. TT allelic frequencies were 62/2892 (2.14%) in subjects with dystonia and 55/3040 (1.81%) in controls. A one-tailed Fisher's exact test failed to confirm nonrandom associations between the TT allele and dystonia ($p = 0.202$). Subgroup analyses for laryngeal dystonia, cervical dystonia, blepharospasm, hand-forearm dystonia, segmental dystonia, multifocal dystonia and generalized dystonia also failed to attain statistical significance in both male and female subjects ($p > 0.06$, for all). Of note, with a Bonferroni correction for multiple comparisons, a *P* value less than 0.05/12 (0.0042)

would be required to maintain a Type I error rate (α) of 0.05 for the subgroup analyses. Using a conservative population prevalence for primary dystonia of 1/10,00012 and an α of 0.05, our case-control cohort had over 95% power to detect a 2-fold relative risk of the TT allele. As seen in Table 2, there was no effect of the TT allele on *THAP1* mRNA expression levels in either leukocytes or lymphoblastoid cell lines. Moreover, there was no statistically significant effect of the TT allele on expression of THAP1 protein (Supporting Information Figure 2). Post-hoc power analysis with an effect size (d) of 0.20 showed that our comparison of leukocyte mRNA expression levels was weakly powered (1 - β = 0.10).

DISCUSSION

THAP1 encodes a 213 residue transcription factor which contains a highly conserved DNA sequence-specific zinc-dependent THAP domain (1–81aa), a proline-rich region, a nuclear localization signal (146–162aa) and a coiled-coil domain.13,14 Overexpression of THAP1 in endothelial cells was used by Cayrol and co-workers15 as an indirect means of identifying THAP1 targets. Down-regulated genes were concentrated in classes related to cell-cycle/cell proliferation and the majority were also regulated by the pRB/E2F pathway. *THAP1* knock-down was associated with decreased expression of 8 pRB/E2F cell-cycle target genes. These data suggest that *THAP1* promoter, UTR and intronic sequence variants associated with alterations in THAP1 expression may exert deleterious effects on neural function and/or neural development and serve as risk factors for dystonia. However, based on the data presented herein, the c.-237_236GA>TT *THAP1* sequence variant has no effect on the risk of developing adult-onset primary dystonia in Caucasians. Moreover, the TT allele does not appear to exert important effects on either transcription or translation in leukocytes and lymphoblastoid cell lines.

In contrast to previous investigations of the c.-237_236GA>TT *THAP1* sequence variant, our study was much larger and focused on late-onset dystonia.4,7 The previously described cohorts from Germany4 and England7 contained a higher percentage of subjects with generalized, segmental and multifocal dystonia. Although the GA>TT variant did not increase overall risk in our cohort of Caucasian subjects, we did not eliminate the possibility that this variant could contribute to dystonia risk in other populations. Similarly, several of our anatomical subgroups may have been underpowered to detect an effect of the TT allele. In this regard, the sole individual homozygous for the TT allele manifests segmental craniocervical dystonia16 with blepharospasm and oromandibular dystonia. Finally, our data do not exclude a role for the TT allele in epistatic interactions with other genes.

Although the results of THAP1 mRNA and protein expression in leukocytes and lymphoblastoid cell lines suggest that the TT allele does not contribute to dystonia risk, our analysis may have been underpowered to detect a small effect. Moreover, it is possible that the effect size of the TT allele on THAP1 expression is developmentally regulated in a tissue specific fashion. Ideally, RNA and protein expression should be examined in several regions of human brain from individuals with GA/GA and GA/TT genotypes at several time points during development. Given the important role for THAP1 in primary dystonia, other sequence variants in promoter, UTR and intronic regions of *THAP1* are also worthy of investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1

Clinical diagnoses, demographics, genotypes and allele frequencies in dystonia and controls

Clinical diagnosis	Number (age of onset)*	Family history†	Minor Allele Frequency (TT)			Genotypes			*P*-value		
			M	F	All	GA/GA	GA/TT	TT/TT	M	F	All
Spasmodic dysphonia	464 (45.8 ± 16.0, 7–85)	7.8%	4/214 (1.9%)	21/714 (2.9%)	25/928 (2.7%)	439/464 (94.6%)	25/464 (5.4%)	0/464 (0%)	0.501	0.082	0.064
Cervical Dystonia	490 (44.5 ± 13.3, 4–76)	9.0%	4/230 (1.7%)	12/750 (1.6)	16/980 (1.6%)	474/490 (96.7%)	16/490 (3.3%)	0/490 (0%)	0.554	0.359	0.420
Blepharospasm	197 (58.1 ± 9.5, 20–73)	6.6%	3/122 (2.5%)	4/272 (1.5%)	7/394 (1.8%)	190/197 (96.4%)	7/197 (3.6%)	0/197 (0%)	0.356	0.416	0.581
Hand-forearm dystonia	52 (35.2 ± 15.9, 7–60)	8.3%	0/46 (0%)	2/58 (3.4%)	2/104 (1.9%)	50/52 (96.2%)	2/52 (3.8%)	0/52 (0%)	0.468	0.317	0.569
Oromandibular dystonia	17 (52.9 ± 11.6, 20–70)	10.5%	0/8 (0%)	0/26 (0%)	0/34 (0%)	17/17 (100%)	0/17 (0%)	0/17 (0%)	0.875	0.607	0.539
Other primary dystonia	36 (42.5 ± 18.3, 10–74)	13.9%	1/28 (3.6%)	1/44 (2.3%)	2/72 (2.8%)	34/36 (94.4%)	2/36 (5.6%)	0/36 (0%)	0.385	0.580	0.382
Segmental Dystonia	140 (47.5 ± 12.2, 12–74)	13.2%	1/92 (1.1%)	6/188 (3.2%)	7/280 (2.5%)	134/140 (95.7%)	5/140 (3.6%)	1/140 (0.7%)	0.551	0.179	0.265
Multifocal Dystonia	24 (32.2 ± 15.8, 7–67)	22.2%	0/16 (0%)	2/32 (6.3%)	2/48 (4.2%)	22/24 (91.7%)	2/24 (8.3%)	0/24 (0%)	0.766	0.133	0.222
Generalized dystonia	26 (23.9 ± 19.4, 1–57)	13.3%	1/24 (4.2%)	0/28 (0%)	1/52 (1.9%)	25/26 (96.2%)	1/26 (3.8%)	0/26 (0%)	0.342	0.584	0.617
Dystonia totals	**1446 (46.2 ± 13.9, 1–85)**	**9.1%**	**14/780 (1.8%)**	**48/2112 (2.3%)**	**62/2892 (2.1%)**	**1385/1446 (95.8%)**	**60/1446 (4.1%)**	**1/1446 (0.1%)**	**0.476**	**0.260**	**0.202**
Neurologically normal controls	1520 (49.3 ± 13.2, 23–83)	NA	22/1320 (1.7%)	33/1720 (1.9%)	55/3040 (1.8%)	1465/1520 (96.4%)	55/1520 (3.6%)	0/1520 (0%)			

*Mean age at study enrollment +/− standard error of the mean (SEM), range (yrs).

†First- or second-degree relative with dystonia. M, male. F, female

TABLE 2

Effect of the c.-237_236GA>TT sequence variant on relative THAP1 expression levels in leukocytes and lymphoblastoid cells

Genotype/phenotype	RNA Leukocytes	RNA Lymphoblastoid cell lines	Protein Lymphoblastoid cell lines
Heterozygous c.-237_236GA>TT Primary dystonia	1.043 ± 0.036* (*n*=20)	0.973 ± 0.065 (*n*=5)	0.811 ± 0.146 (n=5)
Homozygous GA allele Neurologically-normal controls	1.011 ± 0.032 (n=24)	1.020 ± 0.065 (n=10)	1.000 ± 0.074 (n=10)
P-value**	0.508	0.617	0.292

* All values are means ± SEM.

** Difference between dystonia and control samples.