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Substantia Nigra Hyperechogenicity in *DYT6* Dystonia: A Pilot Study

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Keywords

DYT6 dystonia; transcranial sonography; ultrasonography; hyperechogenicity; substantia nigra

DYT6 dystonia is a form of early onset primary dystonia often associated with brachial onset and spread to involve cranial-cervical sites and leg, although the phenotype also includes focal dystonia, and it may start as late as 49 years [1,2]. The recent identification of the *DYT6* gene, *THAP1*, has led to the understanding that *DYT6* dystonia is not restricted to Amish-Mennonites; different mutations have been found in affected individuals of varying ethnic backgrounds, and *DYT6* mutations are the etiology of a significant proportion of familial early-onset dystonia [2,3]. As *THAP1* encodes a transcription factor with potential dopaminergic mechanisms, we performed a pilot study of midbrain transcranial sonography (TCS) in *DYT6* dystonia to evaluate whether there are signal changes in the substantia nigra.

Methods

After giving informed consent, a complete neurological examination, including Burke-Fahn-Marsden Rating Scale (BFM) and determination of sites affected by dystonia, was performed by a movement disorder trained neurologist for eleven dystonia subjects from four families who participated in dystonia genetic studies (Table). Clinical data on all but one patient has been previously reported [1,2,3]. Sixty control individuals, who were recruited from the laboratory or as spouse controls of individuals with movement disorders, and who did not have features of dystonia and parkinsonism were also studied.

TCS examination was performed at either Beth Israel Medical Center or the University of Luebeck, and both centers used the same instrumentation and methodology. One examiner (JH) performed assessment at the University of Luebeck and at the Beth Israel Medical

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Center, and one examiner (KS) performed assessments at Beth Israel Medical Center after joint training. Evaluation was assessed through the preauricular acoustic bone window, using a phased-array ultrasound system with a 2.5-MHz sector transducer (SONOS 5500; Philips, the Netherlands). A standardized axial mesencephalic plane (landmark: butterfly-shaped brainstem) with a maximum depth of 14–16 cm from the temporal bone window on each side was used to localize the maximum extent of the hyperechogenic signals from the ipsilateral SN. The image was zoomed (twofold magnification) and digitally stored on an optical disc for later offline computer-based analysis. Off-line assessment was performed by an independent investigator (NB) who had not been involved in the TCS examination, and was blinded both to gene status and patient and control status. Digitally stored randomized and anonymous images were evaluated using a computer-based analysis (Scion Image Beta 4.02 Win software package) by encircling and measuring the area of hyperechogenicity in the SN (aSN) [4]. The aSNmax was calculated as the greater of the aSN for all subjects with bone windows on either side, and was the aSN for those subjects with bone window on one side.

Mann-Whitney test was performed to compare continuous demographic variables and aSNmax in *DYT6* dystonia subjects and controls, and chi-square tests were used to compare categorical measures. Linear regression was performed to compare aSNmax between *DYT6* cases and controls, adjusting for age and gender (STATA8, Stata, TX).

Results

Table 1 shows the demographic, clinical, mutation and TCS findings for *DYT6* dystonia subjects and controls. At least one transcranial window was obtained for all affected individuals and controls. 97% of controls and ten out of the eleven patients (91%) had bilateral aSN measurements.

Median aSNmax was greater in the *DYT6* subjects [0.29cm² (interquartile range 0.23–0.31cm²)] compared to controls [0.15cm² (interquartile range 0.09–0.18cm²) ($p<0.001$)]. aSNmax differed between groups even after adjusting for age and gender ($p<0.001$). Abnormal echogenicity was defined as an aSNmax of 0.23 cm² or greater, as this represented the upper 5th percentile of controls (Figure). Of *DYT6* subjects, 82% were abnormal compared to 5% among controls ($p<0.001$). Among the *DYT6* subjects, gender, age at disease onset, disease severity (as measured by the BFM), final distribution and type of mutation were not associated with greater hyperechogenicity in post hoc tests.

Discussion

This pilot study suggests that nigral hyperechogenicity is associated with *DYT6* dystonia. Further study is warranted to replicate these findings and to determine whether this is an endophenotype associated with carrying a *DYT6* mutation regardless of disease expression, or whether this is associated developing dystonia. The pathophysiological relationship between nigral hyperechogenicity and *DYT6* dystonia remains elusive, in part because no *DYT6* brains have been examined to date. However, basic and functional imaging studies suggest a potential dopaminergic involvement for THAP1 as it interacts with Par-4 [5], a D-2 dopaminergic receptor modulator, and raclopride PET studies demonstrate reduced striatal D2 receptor availability in *DYT6* gene carriers, both affected and non-manifesting, consistent with loss of D2- bearing neurons [7]. Alternatively, SN hyperechogenicity may represent iron deposition in the SN reticulata or compacta, and may be due to a primary alteration in iron metabolism or non-dopaminergic neuronal degeneration. The latter is supported by the absence of parkinsonian features in *DYT6*. Pathologic evaluation of *DYT6* will shed more light on the etiology of the association. As nigral echogenicity has not been

reported in primary dystonia, future sonographic studies should be extended to include examination of the SN and the other parts of the basal ganglia in other genetic subtypes of primary dystonia.

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Figure.

Table

Clinical and TCS Data

Subject*	Family*	Gender	Age at Exam	Age of onset	BFM motor	Disease distribution	aSN max (cm ²)	aSN mean (cm ²)	Examiner
1	M	M	64	7	68	Generalized	0.31	0.18	KS
2	M	M	49	31	3.5	Focal	0.36	0.26	JH
3	M	F	45	28	3	Segmental	0.18	0.14	JH
4	M	M	19	16	3	Focal	0.29	0.19	JH
5	C	F	45	9	38	Generalized	0.30	0.27	KS
6	C	F	47	21	8.5	Segmental	0.20	0.18	KS
7	9	F	61	12	48	Generalized	0.23	^	KS
8	9	F	40	29	***	Segmental	0.31	0.21	KS
9	2	F	16	2	46.5	Generalized	0.28	0.24	JH
10	2	M	23	3	35.5	Generalized	0.40	0.40	JH
11	****	M	16	11	7.25	Generalized	0.23	0.18	KS
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Subjects (n=11)*,**	45.5%	Men	45 (19-49)	12 (7-28)	22 (3.5-46.5)	54.5% Generalized	0.29 (0.23-0.31)	0.20 (0.18-0.26)	45.5% JH
Controls (n=60)	56.7%	Men	41 (30-51)	---	---	---	0.15 (0.09-0.18)	0.11 (0.0-0.15)	53.3% JH
p-value	NS	p=0.53	NS	---	---	---	p<0.001	p<0.001	p=0.63

* Families M and C harbor the mutation 134_135insGGGT; 137_139deAAC, and clinical features of individuals 1–6 were previously reported by Saunders-Pullman et al (3); Family 9 carries the mutation 51T→A(Ser21Thr), and Family 2 the mutation 134_135insGGGT; 137_139deAAC (2), and clinical features of subjects 7–9 were reported by Bressman et al (2).

Median, Interquartile range, p-value based on comparison of medians

Unable to complete exam for Subject 8

Subject 10 harbored the F45fs73X Amish-Mennonite mutation

^a Adequate bone window was only obtained on one side, therefore mean is not reported for that individual.