

## **Endophenotyping in idiopathic adult onset cervical dystonia**

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## HIGHLIGHTS

- Only multimodal temporal discrimination proves to be an endophenotype of cervical dystonia (CD)
- Multimodal discrimination is abnormal in 36% of healthy 1<sup>st</sup> degree relatives of patients with CD
- Unimodal discrimination, mental rotation and paired pulse TMS are unsuitable endophenotype markers

## **ABSTRACT**

### **Objective**

Idiopathic adult onset cervical dystonia (IAOCD) is considered to be a partially penetrant autosomal dominant genetic condition. Dystonia may result from genetic and environmental factors. In this view, part of the physiology should be an endophenotype stemming from the genetic background. We assessed the most discriminative test to separate patients with IAOCD and healthy controls for further endophenotyping in non-affected 1<sup>st</sup> degree relatives.

### **Methods**

We included patients with IAOCD, their 1<sup>st</sup> degree relatives and healthy controls. Tests performed: 1) Sensory temporal discrimination (visual, tactile, visuo-tactile), 2) Paired pulse paradigms using transcranial magnetic stimulation (TMS), 3) Mental rotation paradigms.

### **Results**

45 patients, 18 healthy controls and 14 non-affected 1<sup>st</sup> degree relatives were recruited. Visuo-tactile temporal discrimination separated best between controls and patients as well as between controls and 1<sup>st</sup> degree relatives. 36% of the latter had an abnormal visuo-tactile temporal discrimination. No difference between patients and healthy controls was found for the other paradigms.

### **Conclusions**

Visuo-tactile temporal discrimination separates controls from patients with IAOCD and its 1<sup>st</sup> degree relatives. 36% of the latter had abnormal visuo-tactile thresholds supporting the role of visuo-tactile temporal discrimination as an endophenotype for IAOCD.

### **Significance**

ur findings expand the understanding of endophenotypes in IAOCD.

**Key words:** dystonia, temporal discrimination, transcranial magnetic stimulation, mental rotation, endophenotype

## 1. INTRODUCTION

Dystonia is a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both. (Albanese et al., 2013)

Idiopathic adult onset cervical dystonia (IAOCD) is considered to be an autosomal dominant genetic condition due to partially penetrant gene/s. (Stojanovic et al., 1995; Waddy et al., 1991)

Hence most of the cases appear to be sporadic with a positive family history in 15-25%. (Waddy et al., 1991) Several genes have been mapped for families, which include members with cervical dystonia (Almasy et al., 1997; Charlesworth et al., 2012; Fuchs et al., 2009; Fuchs et al., 2013; Leube et al., 1996; Valente et al., 2001; Xiao et al., 2012) but in several other families with cervical dystonia linkage to these known genetic loci has been excluded. Like in many disorders, dystonia is considered to be caused by a combination of genetic factors and environmental modifiers. In this view, at least part of the physiology should be a trait stemming from the genetic background. (Hallett 2011)

The identification of the genetic causation has been difficult because informative families are rare due to low penetrance of the phenotype and absence of surrogate markers. One approach to find the gene(s) is to find a sensitive endophenotype, “*a marker of subclinical gene carriage*” in non-affected relatives. A useful model to study endophenotype markers is inherited generalized dystonia (*DYT1*) because penetrance is low. Compared to controls, both, manifesting and non-manifesting *DYT1* gene mutation carriers share common features like deficient inhibition on a cortical level, (Edwards et al., 2003) impaired sensory temporal processing, (Fiorio et al., 2007a) and impaired body movement representation in the mental rotation task. (Fiorio et al., 2008)

Several of these characteristics can also be found in patients with cervical dystonia:

i) *sensory discrimination*: studies have shown that spatial (Molloy et al., 2003; O'Dwyer et al., 2005; Walsh et al., 2007) and temporal sensory discrimination (Bradley et al., 2009; Scontrini et al., 2009; Tinazzi et al., 2004; Kimmich et al., 2014) is impaired in patients with cervical dystonia with the latter being more discriminative. (Bradley et al., 2009; Bradley et al., 2010; Walsh et al.,

2007) Impaired sensory discrimination has been found in 1<sup>st</sup> degree relatives of patients with cervical dystonia (Bradley et al., 2009; Walsh et al., 2007; Kimmich et al., 2014)

ii) *impaired inhibition*: Impaired intracortical inhibition using transcranial magnetic stimulation is present in both hemispheres (ipsi-and contralateral to the dystonic hand) suggesting that this abnormality is likely to be a substrate or trait for dystonia.(Ridding et al., 1995a) In cervical dystonia, there are conflicting reports with reduced short interval intracortical inhibition only when measured from the sternocleidomastoid muscle or only when magnetic stimulation was performed on the contralateral side of head deviation.(Hanajima et al., 1998; Kanovsky et al., 2003)

iii) *The mental rotation paradigm*: Similar to real movements, mentally simulated movements require an intact specific cortico-subcortical motor network(Ionta et al., 2007) as well as intact sensory systems.(Cohen et al., 1996; Vingerhoets et al., 2002) Patients with idiopathic dystonia are slower in mentally rotating corporal objects.(Fiorio et al., 2006; Fiorio et al., 2007b; Fiorio et al., 2008) In patients with focal hand dystonia, slower mental rotation was observed on the affected and unaffected side.(Fiorio et al., 2006)

The aim of this study was to determine the most discriminative test between IA OCD and healthy controls with the idea that an endophenotype marker should be present in subjects presenting with the phenotype and absent in controls. According to the literature we selected temporal sensory discrimination, short intracortical inhibition and mental rotation paradigms. In a second step we applied the test(s) discriminating patients from controls to non-affected 1<sup>st</sup> degree relatives to decide whether it is part of the phenotype or an endophenotype.

## **2. PATIENTS AND METHODS**

We recruited patients with IA OCD who were attending the Movement disorders or botulinum toxin clinics at the National Hospital for Neurology and Neurosurgery, London, their healthy first degree relatives as well as healthy controls without a family history of dystonia. After giving written informed consent, eligible subjects were clinically examined by an experienced Movement disorders specialist (GK). Participants with clinical evidence for polyneuropathy or

carpal tunnel syndrome were excluded. Dystonia severity was quantified with the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS). The study was approved by the local research ethics committee. (Katschnig et al., 2010)

### **2.1. Temporal discrimination thresholds**

Sensory temporal discrimination thresholds (TDT) were assessed by pairs of visual, tactile and visuo-tactile (crossmodal) stimuli according to the published protocol.(Fiorio et al., 2003; Kagi et al., 2013) In short, pairs of stimuli were delivered with progressive increase of the interstimulus interval (ISI). The order of the stimulation paradigms was randomized. Visual stimuli were delivered through light-emitting-diodes (LED) positioned on a black board. Two LEDs in each visual field at 7° from central fixation point. Tactile stimulation consisted of electrical stimuli (constant current) through surface electrodes positioned on the volar side of the index and middle finger of the right and left hand. The anodes were located 1.5cm distally to the cathodes. Stimulation intensity for tactile stimulation was determined for each subject starting at 2mA and an increment of 1mA unless 10 out of 10 stimuli were perceived. Each run started with an ISI of 0ms with progressive increase by steps of 10ms. TDT was considered the first (i.e. the shortest) out of three consecutive ISI at which the two stimuli were recognized as asynchronous. Each run was repeated 4 times with the mean taken as the TDT. Additionally, subjects were asked to judge, which stimulus preceded (or followed) the other. The first (i.e. shortest) out of three consecutive ISI at which subjects also reported correctly the temporal order in a pair of stimuli was termed temporal order judgment (TOJ). Again the mean of 4 runs was taken for further analysis.

### **2.2. Transcranial magnetic stimulation**

Subjects were seated with the hands relaxed (audiovisually controlled) on a pillow. Surface electrodes were placed on the FDI muscle of the dominant hand. Stimulation was performed using 2 Magstim 200 stimulators and a figure-of eight-shaped coil, external wing diameter 9 cm (Magstim, Dyfed, UK). The coil handle was positioned to evoke an anteriorly directed current in

the brain. Optimal coil position was defined as the position with the lowest threshold to evoke a response in the FDI muscle of the contralateral hand. Resting motor threshold was then defined as the intensity to evoke MEPs (>50uV amplitude) in more than 5 out of 10 runs. Active motor threshold was defined likewise but with a minimal background muscle activity (10-15% of maximum). Test MEP intensity was set in order to evoke a MEP of ~1mV. Short intracortical inhibition-/facilitation paradigms followed the protocol described by Kujirai et al.(Kujirai et al., 1993) We used interstimulus intervals of 2 and 3ms for short intracortical inhibition, of 6 and 8ms for intermediate and of 10, 12, 15 ms for intracortical fascilitation. The intensity of the conditioning stimulus was set at 80% of active motor threshold. For short intracortical inhibition-recruitment curve a fixed interstimulus interval of 2ms with increasing intensity of the conditioning stimulus (70%, 80%, 90% of test pulse intensity) was used. The percentage  $((cMEP/tMEP)*100)$  was used for further analysis. Experimental conditions were presented 12 times each.

### **2.3. Mental rotation**

The mental rotation paradigm was adapted from previous studies in idiopathic dystonia.(Fiorio et al., 2006; Fiorio et al., 2007b; Fiorio et al., 2008) In brief, subjects were presented with realistic photos of left or right hands, feet and a head of a woman with a black patch covering either the left or right eye. All three stimuli were presented in six angular orientations (0, 60, 120, 180, 240, and 300) and subjects had to verbally report the laterality of the presented stimuli. Reaction time (RT) was recorded by a microphone and response accuracy was entered into the computer by the researcher. Only RTs with correct laterality judgment were considered for further analysis. The ratio of correct responses to the total number of stimuli was used as error rate.

### **2.4. Statistics**

Data were analyzed using PASW Statistics 20 for Windows and R statistical software (version 2.15.3 (2013-03-01)). For the groups, absolute (n) and relative (%) frequencies are presented for

nominal and mean with standard deviation for continuous patient characteristics.  $\chi^2$  test was applied for gender and handedness and one way ANOVA for age, age of onset, disease duration, handedness, TWSTRS score and subscores I-III.

In a next step, we used a receiver operating characteristic (ROC) curve for the temporal sensory discrimination paradigms to identify the most accurate test to discriminate between patients and healthy controls. The only discriminative test between patients and controls (visuo-tactile paradigm) was further tested by ROC and linear regression analysis between controls and non-affected first degree relatives. To account for possible confounders we included “age” and “education” as co-variables in a linear regression model with “visuo-tactile TDT” as dependent variable and “patient-group” as “factor”.

Because ROC interpretation is difficult with low sample sizes, we added the Mann-Whitney-U test to compare TMS measures (SICI, IM, ICF, SICI<sub>70</sub>, SICI<sub>80</sub>, SICI<sub>90</sub>) between patients and controls.

ANOVA for repeated measures was applied for the mental rotation paradigm. Reaction times were analyzed with one between-subject factor ‘group’ (patients and controls) and three within-subject factors ‘stimulus orientation’ (0, 60, 120, 180, 240, 300), ‘stimulus side’ (right, left) and ‘error rate’.

In this exploratory study the error rate was not controlled at a pre-specified significance level and no correction for multiple testing was considered. However, if appropriate, we provide p-values derived from the corresponding statistical tests and assume that the available data provide evidence against the null hypothesis if the p-value is below 5%.

### **3. RESULTS**

45 patients with cervical dystonia, 18 healthy controls and 14 non-affected 1<sup>st</sup> degree relatives were recruited. There was a significant difference in age between the groups ( $F_{2,7}=.;$   $p<0.0001$ ) due to and non-affected 1<sup>st</sup> degree relatives being younger compared to IA OCD (post-hoc t-test uncorrected ( $p=0.032$  and  $p<0.0001$ ) ( $p=0.0$ ). No significant differences were found for education ( $F_{2,7}=.;$ ), gender ( $p=0.$ ) and handedness ( $p=0.$ ). (Table 1)



### 3.1. Temporal sensory discrimination

The visuo-tactile paradigm was the paradigm discriminating between patients and controls TDT (AUC ) (Figure 1); TOJ (AUC 0.81 [CI<sub>95</sub> 0., 0.]). This was not the case for visual and tactile paradigms; TDT (AUC 0.[CI<sub>95</sub> ] and AUC 0.[CI<sub>95</sub> ]); TOJ (0.[CI<sub>95</sub> ] and AUC 0.[CI<sub>95</sub> ]). (Table 2) Because TOJ data didn't perform better than TDT data we used the latter for further analysis. Calculating J statistics from the visuo-tactile paradigm in healthy controls gives a maximum Youden-Index of 0.at a threshold of with a specificity of % (CI<sub>95</sub> 0., 0.9) and sensitivity of % (CI<sub>95</sub> 0., 0.). At a specificity level of .% sensitivity is 56% (cut off:.). This threshold was reached in five out of 14 (36%) non-affected relatives. ROC analysis of the visuo tactile TDT between controls and non-affected relatives was borderline with an AUC of 0.7 [CI<sub>95</sub> 0.48, 0.85].

The linear regression model with "visuo-tactile TDT" as dependent and "age" and "education" as co-variables revealed a significant difference not only between patients and controls (CI<sub>95</sub> [ , ],  $p < 0.0001$ ) but also between controls and non-affected relatives (CI<sub>95</sub> [0., ],  $p = 0.$ ). Education (years) showed a negative correlation with visuo-tactile TDT (Spearman's  $r$ : -0.234;  $p = 0.033$ ) in patients and controls.

### 3.2. Transcranial magnetic stimulation

Due to recruitment issues only 21 patients and 8 controls could be tested. All patients and 7/8 controls had all tests (sensory testing, TMS, mental rotation). Descriptives of these groups didn't show significant differences except that the patients had 3.7 years less education (14 y vs 17.7 years;  $p = 0.024$ ). Neither ROC curves nor Mann-Whitney U test for SICl, IM, ICF, SICl<sub>70</sub>, SICl<sub>80</sub>, SICl<sub>90</sub> showed differences between patients from controls. (Table 3)

### 3.3. Mental rotation

37/45 patients and 15 controls were studied. . The ANOVA for reaction times was not significant for the factor 'group' ( $F_{1,=}$ ,  $p = 0.$ ) although significant effects were seen for the factor 'degree' ( $F_{5,=}$ ,  $p < 0.00001$ ), 'region' ( $F_{2,=}$ ,  $p < 0.00001$ ) and 'error rate' ( $F_{3,6461} = 6.95$ ,  $p = 0.0084$ ). *Post*

*hoc* tests for 'degree' showed significant differences for 120° ( $p < 0.00001$ ), 180° ( $p < 0.00001$ ) and 240° ( $p = 4$ ) due to longer reaction times for 120° (+ms), 180° (+ms) and 240° (+ms) compared to the reference angle (0°). The differences for the factor 'region' were due to a shorter reaction time for the head (-ms;  $p < 0.00001$ ) compared to the feet. The differences for the factor 'error rate' were due to longer reaction times (+126.6ms) in subjects with errors ( $p = 0.0002$ ) compared to those without.

#### **4. DISCUSSION**

This study confirms previous reports of impaired temporal discrimination in patients with cervical dystonia. (Bradley et al., 2009; Scontrini et al., 2009; Tinazzi et al., 2004; Kimmich et al., 2014; Kagi et al., 2013) 5 out of 14 (36%) non-affected 1<sup>st</sup> degree relatives had abnormal visuo-tactile temporal discrimination thresholds. Considering that only 50% of 1<sup>st</sup> degree relatives are expected to be gene mutation carriers, the finding supports previous reports that subtle sensory abnormalities represent a trait or endophenotype of gene mutation carriage. In addition, Kimmich et al showed that in first degree relatives temporal discrimination exhibits a comparable prevalence compared to the phenotype reaching full penetrance after the age of 48 years. (Kimmich et al., 2014)

The fact that the latter research group found the largest differences in the visual and tactile compared to mixed (visuo-tactile) paradigm is intriguing and hard to explain. (Bradley et al., 2009; Kimmich et al., 2014) (Tinazzi et al., 2002) patients with IA OCD multimodal (visuo-tactile) temporal discrimination might be more affected compared to the unimodal temporal discrimination; i) the parietal lobe as an important center of multimodal sensory integration is part of the dystonia network (Lacruz et al., 1991; Leon and Shadlen, 2003; Neychev et al., 2011; Pastor et al., 2004), ii) this notion receives support by the finding that patients with a good effect to a sensory trick perform significantly better in the visuo-tactile sensory discrimination and that the parietal lobe is activated while performing an effective sensory trick (Kagi et al., 2013; Naumann et al., 2000), iii) the superior colliculi, where multisensory (visual, tactile, auditory)

inputs converge are involved in head-turn generation with its cephalomotor premotor neurons (Hutchinson et al., 2014).

Several previous studies have shown that inhibition is defective within and distant to the affected body region in idiopathic adult onset focal dystonia.(Berardelli et al., 1998; Chen et al., 1997; Hallett2011; Ridding et al., 1995a) In line with most of the literature on short interval intracortical inhibition in cervical dystonia, our study failed to show any difference between patients and controls and therefore doesn't appear to be suitable as endophenotype marker. Due to the fact that the number for controls was rather low (n=8) this notion has to be interpreted with caution. There are several reasons for the variable results reported for short intracortical inhibition in cervical dystonia. For example, intracortical inhibition is reduced by minimal voluntary muscle activation even in muscles remote from the target. Since some patients will have neck movement during assessment this will significantly increase the variability of the patient data.(Ridding et al., 1995b) In addition it should be noted that even in healthy participants, paired-pulse TMS paradigms have an large intersubject variability of up to 80%.(Wassermann 2002)

The mental rotation paradigm didn't separate patients from controls. The ANOVA model failed to confirm the differences found between patients and healthy controls in previous studies using the same paradigm, methodological setting and with the same statistics.(Fiorio et al., 2007b; Katschnig et al., 2010) Compared to these two studies the patients were within the same range but the controls performed worse. Since only controls but not patients performed worse, it makes methodological reasons unlikely to account for the differences. For the purpose of our study, mental rotation does not represent a valuable endophenotype marker.

*What is the value of temporal sensory discrimination testing in 1<sup>st</sup> degree relatives for genetic testing?*

Although subtle sensory abnormalities seem to represent a trait of dystonia, its value for genetic studies is limited. Imagine we are investigating one family with dystonia in whom there are 5 affected members. For genetic studies we would need another 5 non-affected first degree

relatives who are correctly selected (true positives) by a given test. In brief, to get 5 true positives we would need to test 20 first degree relatives (because we expect 10 to be non-gene mutation carriers due to the autosomal dominant inheritance). It is important to set the specificity near 100% because in genetic linkage studies the required “*logarithm of odds*” (LOD) score of  $>+3$  (evidence for linkage) is achieved in 10 true positive samples. If 1 sample is a false positive the LOD score decreases to around +1.5. In our 14 non-affected first degree relatives (we assume that half (7) are mutation carriers) we would have recruited 5 subjects with the specificity set at 95.7%.

In conclusion, only crossmodal (visuo-tactile) temporal sensory discrimination separated healthy controls from patients as well as from their non-affected 1<sup>st</sup> degree relatives. Almost 40% of the latter had abnormal visuo-tactile temporal discrimination thresholds supporting its role as an endophenotype marker for IA OCD. Unimodal (visual, tactile) temporal discrimination, intracortical inhibition/facilitation or mental rotation did not separate patients from healthy controls making them unsuitable for endophenotype markers.

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## TABLES AND FIGURES

**TABLE 1: Patient characteristics**

	IAOCD	1 <sup>st</sup> ° relatives	controls
number	45	14	23
gender (% females)	64.4	75	56.5
handedness (% right)	86.7	100	95.7
age (y)	56.3 ± 10.9	38.7 ± 14.7	48.9 ± 16.8
age of onset (y)	41.5 ± 14.1	---	---
education (y)	14.2	14.9	16.7
disease duration (y)	14.5 ± 10.3	---	---
TWSTRS			
total	23.9 ± 10.4	---	---
subscore I	11.8 ± 3.9	---	---
subscore II	7.7 ± 4.2	---	---
subscore III	5.2 ± 4.7	---	---

*IAOCD: idiopathic adult onset cervical dystonia. TWSTRS: Toronto Western Spasmodic Torticollis Rating Scale. Data are presented as mean with standard deviation if not stated otherwise*



**TABLE 2: Temporal discrimination thresholds**

	IAOCD	1 <sup>st</sup> ° relatives	controls	ROC, AUC [CI(95%)] IAOCD vs controls
n	45	14	23	
visual tdt	54.2 ± 18.2	40.6 ± 10.8	42.4 ± 16.2	0.68 [0.54; 0.82]
visual toj	56.1 ± 18.7	43.5 ± 12.6	44.8 ± 17.0	0.68 [0.54; 0.82]
tactile tdt	89.6 ± 42.9	70.1 ± 27.5	63.7 ± 27.9	0.69 [0.56; 0.83]
tactile toj	100.0 ± 51.2	79 ± 33.5	71.7 ± 32.2	0.67 [0.53; 0.81]
cm tdt	140.6 ± 49.5	115.8 ± 35.8	89.8 ± 25.3	0.85 [0.76; 0.94]
cm toj	146.1 ± 49.6	127 ± 31.1	99.9 ± 30.9	0.81 [0.70; 0.92]

*IAOCD: idiopathic adult onset cervical dystonia. cm: crossmodal (visuo-tactile), tdt: temporal discrimination threshold in milliseconds, toj: temporal order judgment in milliseconds, CI: confidence interval, ROC: Receiver operating characteristic, AUC: area under the curve. The values are reported as mean with standard deviation.*

**TABLE 3: Short intracortical inhibition (SICI), intracortical facilitation (ICF)**

	IAOCD	controls	p-value*
n	21	8	
SICI 70%	81.1 $\pm$ 33.1	103.1 $\pm$ 67.6	0.340
SICI 80%	63.6 $\pm$ 32.9	72.2 $\pm$ 28.5	0.374
SICI 90%	63.6 $\pm$ 55.1	52.6 $\pm$ 26.5	0.783
SICI	49.6 $\pm$ 26.2	53.4 $\pm$ 37.1	0.850
Intermediate	102.9 $\pm$ 44.4	101.4 $\pm$ 30.2	1
ICF	122.7 $\pm$ 56.7	126.1 $\pm$ 41.4	0.613

*IAOCD: idiopathic adult onset cervical dystonia. SICI: short intra-cortical inhibition, ICF: intracortical facilitation. The values are presented as mean with standard deviation. \*Mann-Whitney-U-Test*

**Supplemental Table 1: Mean reaction times (ms) in patients and controls for the three body-stimuli (hand, feet, head), and their laterality (left and right) and orientations (0°, 60°, 120°, 180°, 240°, 300°)**

	Right						Left					
	0°	60°	120°	180°	240°	300°	0°	60°	120°	180°	240°	300°
<b>Hand</b>												
IAOCD	2118	2266	2586	2773	2071	1907	2082	1960	2036	2670	2276	2091
controls	1768	1915	2126	2332	1843	1848	1881	1644	1922	2260	2088	1846
<b>Head</b>												
IAOCD	1847	1741	1938	1957	1813	1814	1770	1789	1827	2225	1726	1768
controls	1659	1553	1741	1965	1793	1627	1481	1451	1590	1791	1564	1446
<b>Feet</b>												
IAOCD	2039	2042	2391	2565	2337	2109	2003	2153	2390	2550	2114	2042
controls	1926	1785	2117	2082	2033	1876	1688	2064	2067	2492	1978	1864

IAOCD: idiopathic adult onset cervical dystonia. ms: milliseconds.

**Figure 1:**

Title: ROC curves for different temporal discrimination modalities in patients with IA OCD vs. healthy controls

Legend: *tactile\_tdt: tactile temporal discrimination thresholds, visual\_tdt: visual temporal discrimination thresholds, crossm\_tdt: crossmodal (visuo-tactile) temporal discrimination thresholds*