Comparing Endophenotypes in Adult-Onset Primary Torsion Dystonia

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Abstract: Adult-onset primary torsion dystonia (AOPTD) has an autosomal dominant pattern of inheritance with markedly reduced penetrance; the genetic causes of most forms of AOPTD remain unknown. Endophenotypes, markers of subclinical gene carriage, may be of use detecting non-manifesting gene carriers in relatives of AOPTD patients. The aim of this study was to compare the utility of the spatial discrimination threshold (SDT) and temporal discrimination threshold (TDT) as potential endophenotypes in AOPTD. Data on other published candidate endophenotypes are also considered. Both SDT and TDT testing were performed in 24 AOPTD patients and 34 of their unaffected first degree relatives; results were compared with normal values from a control population. Of the 24 AOPTD patients 5 (21%) had abnormal SDTs and 20 (83%) had abnormal TDTs. Of the 34 first degree relatives 17 (50%) had abnormal SDTs and 14 (41%) had abnormal TDTs. Discordant results on SDT and TDT testing were found in 16 (67%) AOPTD patients and 21 (62%) first degree relatives. TDT testing has superior sensitivity compared to SDT testing in AOPTD patients; although false positive TDTs are recognised, the specificity of TDT testing in unaffected relatives is not determinable. The high level of discordance between the two tests probably relates methodological difficulties with SDT testing. The SDT is an unreliable AOPTD endophenotype; TDT testing fulfils criteria for a reliable endophenotype with a high sensitivity. © 2009 Movement Disorder Society

Key words: AOPTD; basal ganglia; endophenotype; temporal discrimination; spatial discrimination

INTRODUCTION

The original description of an endophenotype dates from the early 1970s1 when it was first applied in psychiatry to assist in the investigation of complex genetic disorders such as schizophrenia. It was proposed that the phenotypes of disorders such as schizophrenia are so variable and dependant on so many interacting genetic derangements that routine evaluation of patients could never lead to successful gene identification. An endophenotype may be considered a subclinical marker of genetic liability to a disorder, whether this is determined by carriage of a single gene mutation or a number of genetic risk factors. They are biomarkers (defined as any disease-associated biological finding) that fulfil a number of specific criteria which are designed to determine that the marker is associated with the presence of the gene rather than simply manifestation of the disease state. Examples of endophenotypes in the literature include laboratory measurements, such as copper studies in Wilson’s disease; physiological testing abnormalities, for example the specific EEG findings in juvenile myoclonic epilepsy2; or imaging findings, including the specific pattern of MRI white matter change in CADASIL.3

Endophenotypes could be used in linkage studies to identify genetic loci in poorly penetrant disorder; a number of criteria for a proposed endophenotype exist4–6; the endophenotype should be associated with the disease under investigation in the general popula-
tion, a heritable trait transmitted with disease in pedigrees, a finding that is “state-independent” (i.e. unaffected by disease expression or treatment) and should have a higher frequency amongst unaffected relatives in pedigrees than in the general population. An ideal endophenotype for an autosomal dominant disorder should be abnormal in all affected patients, half of unaffected first degree relatives and no control subjects.

Adult-onset primary torsion dystonia (AOPTD) is the commonest form of dystonia and is considered to be a genetically determined disorder with autosomal dominant transmission.7,8 The considerably low penetrance (in the region of 12–15%) results in a paucity of informative multiplex families; the majority of cases may appear sporadic in nature but up to 25% have a family history on detailed evaluation.9 Although a number of loci and genes have been identified,10 the genetic causes of most AOPTD phenotypes remain unknown. A sensitive endophenotype would increase the number of subjects available for genetic studies. Sensory abnormalities in AOPTD include abnormal spatial discrimination, temporal discrimination, and vibration-induced illusion of movement.11–18 It is hypothesised that a disorder of sensory integration possibly involving the basal ganglia is the cause of these sensory abnormalities. Given the evidence of a genetic disorder, there has been significant investigation of candidate sensory endophenotypes.

Endophenotypes have been particularly studied in DYT1 dystonia because of its incomplete penetrance, thus a potential endophenotypic trait in a can be validated in nonmanifesting carriers of the GAG deletion in TorsinA. In addition, abnormalities demonstrated in nonmanifesting DYT1 carriers17,19–22 support the hypothesis that the physiological abnormalities of sensory processing seen in dystonia result from genetic determinants rather than secondary changes induced by the movement disorder.

The spatial discrimination threshold (SDT) is determined using a grating orientation task employing Johnson-van Boven-Philips (JVP) domes applied to the fingertip. Abnormal SDTs have been found in AOPTD patients as well as their unaffected relatives.12,15,16,23 In addition to disordered sensory processing in the basal ganglia, abnormal representation in the primary sensory cortex (S1) may be important in the causation of SDT abnormalities.13,24 Plasticity in S1 may explain some of the variability of SDT results, including improvement with botulinum toxin treatment.25

The temporal discrimination threshold (TDT) is defined as the shortest time interval at which a subject can determine that two stimuli are asynchronous. Abnormal TDTs have been described in a number of conditions including DYT1-dystonia,17 writer’s cramp,18,26 blepharospasm,26,27 Parkinson’s disease,28,29 and multiple system atrophy30 and as such may be an indicator of abnormal basal ganglia function. Functional imaging studies have demonstrated activation of the basal ganglia and other subcortical structures during a TDT task; higher cortical activity specific to TDT (not seen in SDT testing) is found in the anterior cingulate and presupplementary motor area, these regions may be involved in the interpretation of timing information.31 In contrast, the basic timekeeper appears to be the putamen, where the earliest activation occurs during encoding of time intervals32 and dopaminergic pathways may be particularly important.33 It has been demonstrated that easier TDT tasks induce greater putaminal activation than difficult TDT tasks (i.e. stimuli presented near the threshold for simultaneity perception) when additional areas are activated.34 In this way the putamen seems to act as the automatic time keeper in low-attention situations.

In addition to abnormal SDT and TDT, a number of other candidate endophenotypes have been investigated in AOPTD including abnormalities in vibration-induced illusion of movement (VIIM),35 Positron Emission Tomography (PET),19 Diffusion Tensor Imaging (DTI),21 and transcranial magnetic stimulation (TMS).36

In this article, we compare the utility of two sensory tests (SDT and TDT) as potential endophenotypes in AOPTD.

PATIENTS AND METHODS

Patients

Both SDT and TDT testing were performed in 24 AOPTD patients (14 cervical dystonia, 10 writer’s cramp) (15 sporadic, 9 familial)(mean age 52 years, range 34–63 years) and 34 of their unaffected first-degree relatives (22 of familial and 12 of sporadic AOPTD patients) (mean age 42 years, range 26–69 years). The normal control subjects were the 141 control subjects in a published SDT study15 and the 43 control subjects in a published TDT study.36 Informed consent was obtained; the study was approved by the Ethics and Medical Research Committee, St. Vincent’s University Hospital, Dublin.

Methods

The SDT and TDT testing of individual patients and relatives were performed by two separate trained examiners (SDT by RW, TDT by DB) without knowl-
SDT was examined using JVP domes as described previously. TDTs were examined as described previously and were measured for three tasks: (1) visual–visual, (2) tactile–tactile and (3) visual-tactile. Data from the three conditions were averaged to obtain the combined TDT score. A brief description of testing procedures is provided (Supporting Information 1). We divided the 141 control subjects for SDT into four age bands (20–29, 30–39, 40–49 and 50–65 years). The $Z$-Scores calculated for each individual (AOPTD patient or relative) are based on the normal values found in control subjects from their respective age band in the control group. Similarly, we divided the controls for TDT testing into two age bands (under 50, over 50). This approach controls for the effect of age, particularly for SDT examination. We defined a normal cut-off of 2.5 standard deviations above the relevant age-related control group mean. $Z$-scores of equal to or greater than 2.5 were considered abnormal.

RESULTS

Spatial Discrimination Thresholds

The SDT $Z$ scores in the 141 control subjects ranged from $-2.06$ to $+2.63$; one control subject exceeded the upper limit of normal. Abnormal SDTs ($Z$ score $>2.5$) were found in 5 of 24 (21%) AOPTD patients and in 17 of 34 (50%) first degree relatives (Fig. 1). Original results are provided (Supporting Information 2).

FIG. 1. Comparison of the frequencies of abnormalities in SDT and TDT testing. In AOPTD patients, abnormal SDTs were found in 5/24 (21%) and abnormal TDTs in 20/24 (83%). In unaffected relatives, abnormal SDTs were found in 17/34 (50%) and abnormal TDTs in 14/34 (41%). 1/141 control subjects had an abnormal SDT result and none of the 43 control subjects had an abnormal TDT. Control results for TDT were more closely grouped and the spread of abnormal results was greater than that seen with SDT testing.

Temporal Discrimination Thresholds

All of the 43 control subjects’ $Z$-scores were less than 2.5 (range $-2.21$ to $+1.76$). Abnormal TDTs ($Z$ score $>2.5$) were found in 20 of 24 (83%) AOPTD patients and 14 of 34 (41%) of first degree relatives (Fig. 1). Original results are provided (Supporting Information 2).

The frequencies of SDT and TDT abnormalities were similar in Cervical Dystonia and Writer’s Cramp patients (Supporting Information 3).

SDT and TDT Testing Compared

In the 24 AOPTD patients there were concordant results in 8 (33%) patients; in 4 patients both tests were abnormal and in 4 both tests were normal. In 16 (67%) of the 24 AOPTD patients there were discordant results; 1 patient had a normal TDT with an abnormal SDT and in 15 patients the TDT was abnormal but the SDT was normal. In relation to phenotype, results were concordant in 3/14 (22%) cervical dystonia patients and 5/10 (50%) writer’s cramp patients; this difference was not statistically significant (Fisher’s exact test; $P = 0.204$).

In the 34 unaffected first degree relatives, 13 (38%) had concordant findings; in 4 patients both tests were abnormal and in 9 both were normal. In 21 (62%) of the 34 relatives the results were discordant; 9 relatives had an abnormal TDT with a normal SDT and 12 relatives had a normal TDT with an abnormal SDT (Fig. 2).

FIG. 2. Scatterplot of TDT $Z$-Score vs. SDT $Z$-Score in 58 subjects who had both tests (24 AOPTD patients and 34 unaffected relatives). Overall 36% (21/58) subjects were concordant (both normal or both abnormal). The majority of discordant results (24/37) represented subjects with normal SDT and abnormal TDT, possibly reflecting the lower sensitivity of SDT testing. 13 subjects had abnormal SDT results with a normal TDT and these may represent false positive SDT abnormalities.
DISCUSSION

In our AOPTD patients we found a remarkable level of discordance (67%) between the SDT and TDT test results. In the unaffected first degree relatives, although both tests were abnormal in a significant proportion (SDT 50%, TDT 41%), there was again a notable discordance of 62%. Clearly one of these two potential endophenotypes is more unreliable than the other. The frequencies of abnormalities in our AOPTD patients (SDT 21%, TDT 83%) indicate that TDT is a more sensitive marker of abnormal sensory processing in AOPTD. Moreover, in control subjects the distribution of TDT results was narrower (range −2.21 SD to +1.76 SD) than the SDT control range (range −2.06 SD to +2.63 SD) suggesting greater confidence that an abnormal result is indicative of abnormal central sensory processing. Furthermore, as can be seen from Figure 1, the range of abnormal Z-scores for the TDT is much greater than that of the SDT.

The SDT is relatively sensitive to age related changes in the peripheral nervous system; a number of discordant results may thus be due to the lower specificity of SDT testing. There is marked increase in the sensory threshold with age which reflects the natural effect of age on the peripheral nervous system. This age effect renders it impossible to determine with accuracy the upper limit of normal of the SDT over the age of 65 and probably limits sensitivity of the test over the age of 50. This variation in the SDT sensitivity with age might partly (but not completely) explain why AOPTD patients (who had a mean age of 52 years) had fewer abnormal SDT results than their unaffected first degree relatives (mean age 42 years).

SDT has more potential for error due to the variability in stimuli presented to subjects using manually applied JVP domes in comparison with the electronically-determined electrical stimuli in the TDT testing procedure. The basal ganglia, and dopaminergic pathways in particular, play a particular role in timekeeping in the CNS. Thus the TDT may be a more sensitive measure of the postulated dopaminergic dysfunction in AOPTD patients.

Validation of TDT as an Endophenotype

There is no gold standard with which to validate any candidate endophenotype in AOPTD as the genotype is not known. TDT has been examined in other genetic forms of dystonia. Fiorio and colleagues found that TDT was abnormal in DYT1-carriers (both manifesting and nonmanifesting) compared with noncarrier relatives and healthy controls. In addition, in PINK1 PARKinsonism they found abnormal TDTs in both homozygous manifesting patients and heterozygous nonmanifesting relatives compared with controls.

An ideal endophenotype for an autosomal dominant disorder should be detected in 100% of affected patients, approximately 50% of unaffected first degree relatives and no healthy controls. A review of the results for TDT, SDT and published work on other endophenotypes indicates that the frequencies of abnormalities in patients, relatives and controls for TDT are compatible with a useful endophenotype in AOPTD (Table 1), although this should be validated in further studies in other populations.

Using Voxel-Based Morphometry, it has been demonstrated that, in comparison to relatives with normal TDTs, unaffected relatives with abnormal TDTs share a structural abnormality, bilateral putaminal enlargement, with AOPTD patients. Thus TDT is supported as a valid endophenotype in AOPTD by its association with a recognised pathological finding in AOPTD. A recent paper suggests putaminal enlargement is a primary feature of adult onset dystonia and provides evidence that, in DYT1 dystonia, it is due to both gene and disease manifestation effects. Further validation of the TDT as an endophenotype comes from a study of multiplex AOPTD families in which an obligate carrier (an unaffected sibling member with an affected sibling and an affected child) examined by TDT had an abnormal Z score of 9.4. In the same study, autosomal dominant transmission of abnormal TDTs was demonstrated in the multiplex pedigrees across two generations and no parents with normal TDTs had offspring with abnormal TDTs.
ALTERNATIVE ENDOPHENOTYPES IN AOPTD

Candidate AOPTD endophenotypes include abnormalities in SDT, TDT, VIIM, PET, and TMS. The relative advantages and disadvantages of these techniques as potential endophenotypes have been examined in a number of patient populations.

Vibration-Induced Illusion of Movement (VIIM)

Vibration of a muscle through stimulation of the muscle spindle can induce an illusion of movement. This perception is reduced in AOPTD patients. VIIM abnormalities were examined in a cohort of 30 AOPTD patients, 57 relatives and 19 controls. VIIM abnormalities were found in 80% of AOPTD patients and approximately 60% of first degree relatives. As an endophenotype VIIM is not ideal, given that abnormalities were found in 21% of control subjects and thus it has a sub-optimal specificity and positive predictive value.

Transcranial Magnetic Stimulation

Inhibitory mechanisms in the central nervous system are abnormal in patients with dystonia. Transcranial magnetic stimulation has been used to assess intracortical activity in DYT1 dystonia. Edwards and colleagues studied manifesting DYT1 patients, nonmanifesting DTY1 carriers and controls. They reported reduced intracortical inhibition with reduced cortical silent periods in DYT1 carriers, regardless of phenotype expression, which is compatible with the reduced GABAergic activity postulated in dystonia. Their findings may be explained by secondary change resulting from a primary basal ganglia disorder.

Positron Emission Tomography

Eidelberg and colleagues have shown using PET that metabolism is diffusely altered in DYT1 dystonia. In both manifesting and nonmanifesting DTY1 carriers metabolism was shown to be increased in the lentiform nucleus, cerebellum, and supplementary motor area. Additional abnormalities were seen in manifesting subjects only, including hypermetabolism in the midbrain and thalamus. Further PET studies have examined other dystonia-related mutations, including the less common DYT6 dystonia linked to 8q21–22. Compared to DYT1 dystonia, similar metabolism was seen in DYT6 carriers, both manifesting and nonmanifesting. Changes specific to DYT1 included hypermetabolism in the putamen, anterior cingulate and cerebellar hemispheres, while DYT6 patients had hypometabolism in the putamen and hypermetabolism in the temporal lobe. PET has also been used to examine motor learning in nonmanifesting DYT1 carriers and reported increased metabolic activity during both sequence learning and motor execution compared with controls. PET with [11C] raclopride (RAC) scanning has been used to examine D2 receptor availability in the basal ganglia and selected extra-striatal regions in DYT1 and DYT6 carriers. The authors found that both DYT1 and DYT6 mutation carriers had reduced receptor availability in caudate, putamen and ventrolateral thalamus compared to controls. The relatively consistent patterns of abnormalities relating to particular genotypes and phenotypes along with some penetrance-related findings are the basis for the proposed use of functional imaging as an endophenotype in AOPTD. This modality also provides significant insight into the pathogenesis of the disorder.

CHOICE OF ENDOPHENOTYPE

In many published endophenotype studies, group results are presented so that, while significant differences are demonstrated between affected individuals, relatives and controls, it is not possible to be certain of the status of any one individual (Table 1). TDT testing appears capable of assigning status to individuals. TDT is not without limitations; false negative and false positive results occur. In this study 4 of the 24 AOPTD patients had normal TDTs. Furthermore, as part of an ongoing genetic study in our department, we found that removing one unaffected relative with an abnormal TDT (Z-Score 6.6) from a linkage analysis resulted in a significant increase in the LOD score to greater than +3.0 (unpublished results). A false positive TDT was found in the control group in a study of TDT in
PINK1; one of the control subjects had a TDT greater than the chosen cut off for normal of 2 standard deviations above the control mean. Overall however, the number of inappropriate results seems to be low. It is of critical importance that an endophenotype misclassifies the fewest possible individuals as incorrect assignments in a linkage analysis can negatively affect the outcome. One example of this relates to a Swiss family with dopa-responsive dystonia incorrectly assigned to DYT1. In addition, while TDT appears to be relatively sensitive in detecting subclinical basal ganglia dysfunction, it is not specific to AOPTD because abnormal TDTs are seen in other basal ganglia disorders. A number of proposed AOPTD endophenotypes do not reliably dichotomise unaffected relatives to allow assignment of probable gene carriage. Based on the available evidence TDT testing satisfies the criteria for a useful endophenotype.

Acknowledgments: This research was funded by Dystonia Ireland, a non-profit patient support charity, (www.dystonia) and by the Health Research Board of Ireland (www.hrb.ie).

Author Roles: David Bradley recruited participants, designed the TDT testing protocol, performed TDT testing, analysed data and wrote the manuscript. Robert Whelan designed the TDT testing protocol, analysed data, and edited the manuscript. Richard Walsh recruited participants, performed SDT testing and edited the manuscript. John O’Dwyer designed the SDT testing protocol, recruited participants and reviewed the manuscript. Richard Reilly reviewed the manuscript and provided mentorship. Siobhan Hutchinson recruited participants and reviewed the manuscript. Fiona Molloy recruited participants and reviewed the manuscript. Michael Hutchinson designed the study, edited the manuscript, and provided mentorship.

Financial Disclosures: R. Reilly and R. Whelan have been awarded funding from Enterprise Ireland, a state finding agency. M. Hutchinson sits on the medical advisory board of Biogen Idec. None of the authors have any further financial disclosures to make under the headings of stock ownership in medically-related fields, consultancies, advisory boards, partnerships, honoraria, grants, intellectual property rights, expert testimony, employment, contracts, or royalties.

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