Electronic letter

No evidence of allelic heterogeneity in the DYT1 gene of European patients with early onset torsion dystonia

Sylvie Tuffery-Giraud, Laurent Cavalier, Agathe Roubertie, Caroline Guittard, Soukeyna Carles, Patrick Calvas, Bernard Échenne, Philippe Coubes, Mireille Claudes

Editor—Torsion dystonia is a movement disorder characterised by sustained involuntary muscle contractions, frequently causing twisting and repetitive movements or abnormal postures. Primary torsion dystonia (PTD) occurs either in a familial or sporadic pattern with dystonia as the sole phenotypic manifestation with the exception that tremor can be present as well. Early onset, generalised torsion dystonia is the most severe form of hereditary dystonia, and the most prevalent form is the result of mutation in the DYT1 (TOR1A) gene on chromosome 9q34. Inheritance follows an autosomal dominant mode of transmission with reduced penetrance (30-40%), and there is a particularly high prevalence in Ashkenazi Jews (AJ) as a result of a founder effect and genetic drift. Early onset primary dystonia resulting from DYT1 usually starts in an arm or a leg at a mean age of 12.5 years (this can range, however, from 4 to 44 years). More than 60-70% of cases have progression to generalised dystonia involving limb and axial muscles, but the cranial muscles are only involved in 11-18% of cases. The causative mutation has been identified as a 3 bp deletion (946delGAG) in the coding sequence of the DYT1 gene, resulting in loss of one of a pair of glutamic acid residues near the C-terminus of the encoded protein, torsinA. Presumably, deletion of this amino acid results in a critical change in the function of the gene product that leads to clinical signs of dystonia.

Currently, this mutation is the only sequence change found to be associated with the disease state, regardless of ethnic origin, both as an inherited or a de novo deletion. The ΔGAG in the heterozygous state accounts for 50-60% of non-Jewish (NJ) subjects and over 90% of AJ subjects with early, limb onset generalised dystonia. In contrast to the AJ population, analysis of haplotypes in the NJ population suggests no founder effect but multiple events resulting in independent occurrences of the same recurrent mutation, a situation which is highly unusual in clinical genetics.

The high proportion of European PTD patients with early limb onset phenotype who do not carry the mutation (40%) may represent either allelic or locus heterogeneity in dystonia.

Whether other changes within the DYT1 gene lead to dystonia or some other phenotype still remains unknown, as patients with early onset PTD have been tested only for the 946delGAG mutation in recent studies. Only one previous study reported screening for new DYT1 mutations using a genomic DNA approach in American dystonic patients. The main purpose of this study was to assess if non-Jewish European patients with early onset torsion dystonia without the deletion had other mutations in the DYT1 gene or anomalies in other genes. Additional mutations in the DYT1 gene were screened by transcript analysis to allow detection of base substitutions as well as rearrangements resulting from splicing defects, or heterozygous exon deletion (or duplication) not detected by simple PCR amplification.

Patients, methods, and results

Most of the families in our series were ascertained through probands with early onset generalised dystonia diagnosed and treated by deep brain stimulation in the Department of Paediatric Neurosurgery at the Montpellier University Hospital. Additional patients were referred from Neuropaediatrics or Clinical Genetics services. Therefore, our series of patients did not represent a random sample of PTD, but mostly included patients with severe, generalised PTD and/or early onset, in infancy or childhood. The diagnosis of PTD was established according to current criteria. A total of 35 patients were selected for the study (10 familial index cases and 25 sporadic cases). All had onset of symptoms before the age of 24. A very early onset (≤5 years) group of 11 NJ patients was observed. All the patients were French except one of Turkish origin; two patients had one AJ parent. All families gave informed consent before participating in the study.

The cohort of 35 probands was first examined for the recurrent 946delGAG mutation in the DYT1 gene. PCR products were generated from genomic DNA with primers 6419 and H48, and the 200 bp product was digested with RsRI of which one cutting site is abolished by the GAG deleted sequence. Taken together, 14 subjects (14/35, 40%) were
positive for the GAG deletion in the *DYT1* gene, including the two patients whose fathers were of AJ origin. Fifty seven percent (8/14) were males. The mean age at onset of the group was 8.8 years (SD 2.56) (range 6-15 years). The term “typical *DYT1* phenotype” is used generally for a selected group of patients characterised by early, limb onset, generalised dystonia without spread to the cranial muscles such as the face, pharynx, or tongue. Any patient who conformed to this phenotype showed the GAG deletion, whereas only two patients (2/17 = 11.8%) with early, limb onset, generalised PTD spreading to cranial muscles tested positive for the *DYT1* mutation. The mutation was also not found in patients with focal dystonia nor in any patient with onset of symptoms in the neck, trunk, or face. Furthermore, all patients with early childhood onset (<5 years) of dystonia were non-carriers. This finding agrees with recent published guidelines for diagnostic testing, which states that the optimal classification rule to predict carriers in the NJ population uses for criteria an age at onset between 6 and 16 years.  

Nine patients (64%) had at least one relative affected by PTD (table 1). It is noteworthy that in three cases (patients 2, 3, and 13), the affected relatives suffering from either torticollis or writer’s cramp had not received a diagnosis of PTD until they underwent detailed clinical examination by neurologists as part of this study. A wide spectrum of symptoms that can overlap with other forms of dystonia was observed in the affected relatives, and disease severity varied considerably within the same family (table 1). Moreover, the father of patient 7 was found to have blepharospasm, an atypical clinical presentation of the *DYT1* mutation. *DYT1*-related dystonia does not commonly affect facial muscles as the first site of onset, and such a phenotype has not been previously reported in *DYT1* carriers. This patient is currently treated with botulinum toxin.

All available affected subjects were screened for the 946delGAG mutation in these nine families and were found to carry the *DYT1* mutation (table 1). Asymptomatic *DYT1* carriers were also identified in families of patients 2, 3, 4, and 8, which was consistent with the assumption of autosomal dominant inheritance with reduced penetrance (table 1). Genotypes were determined for four polymorphic markers (D9S2160, D9S2161, D9S63, and D9S2162) surrounding the *DYT1* gene in all carriers of the deletion and available relatives. Products were analysed by GeneScan analysis on an ABI 377 sequencer with 672 software. No common haplotype could be established between *DYT1* carriers, indicating that the GAG deletion has arisen repeatedly but independently in the studied population.

The remaining five positive deletion patients were apparently sporadic cases. In one case, both parents were available for genetic testing and the mutation was found in the unaffected patient’s mother. For each of the four other cases (including the two patients of AJ ethnic background), only the mother could be analysed, and was shown to be GAG deletion negative; the father should therefore be tested to determine if these sporadic cases result from either de novo mutation or low penetrance of the mutant gene.  

Twenty one patients (21/35 = 60%) with early onset PTD did not carry the GAG deletion. Many of these early onset non-carriers share other clinical features with carriers, including limb onset and a tendency to spread to other body regions. We investigated the possibility of other mutations in *DYT1* producing an early onset phenotype in these patients by screening *DYT1* transcripts from peripheral blood lymphocyte RNA for additional mutations. New blood samples for RNA isolation were obtained in 16 of the 21 non-deleted patients (table 1). None of them was of AJ origin. The age at onset varied more widely in this group compared with the *DYT1* positive group (mean age of 7.4 years (SD 5.81)). Among the 16 patients under investigation (table 1), 14 had generalised disease with involvement of cervicocranial muscles. Ten of them had limb onset whereas three of them had onset in the trunk, neck, or face (cases 30, 31, and 32). In case 33, the first symptom was dysarthria. Patients 34 and 35 developed dystonia of the right upper limb, but no further spread of dystonia had occurred at the time of examination. All but one (patient 34) were sporadic cases. Familial presentation of dystonia in case 34 should be noted. The sister of this patient was reported to have transient idiopathic focal dystonia at the age of 3 years, which resolved rapidly within five months. She is currently aged 8 years and does not present any sign of dystonia.

An 1108 bp region including the 998 bp of the coding sequence was PCR amplified from cDNA derived from lymphocytes using the Access RT-PCR system (Promega, Madison, WI) and specific gene primers (5'-TTCGCGCCGGAAGACA-3', forward, and 5'-GTGGAAAGGACTGATGTGTTTTC-3', reverse). The PCR products were then submitted to a second round of amplification generating two overlapping fragments by using the following primers (5'-GCAAGGGTGGGGGCGGTCC-3' and 5'-AAAGGGTTAGGGGATCTGA TGAG-3' for fragment 1) and (5'-TGTAACAG GATCAGTACAGTTGTG-3' and 5'-GATGTT TTCTTTCAACTCAAGGC-3' for fragment 2). Both fragments were screened for heterozygous mutations by sequence analysis. We detected no nucleotide change that would alter the amino acid sequence of the protein in any of the 16 patients analysed. In addition, we did not find any deletion (or duplication) of a single exon or aberrantly spliced transcripts indicative of splicing mutations. In five out of the 16 patients who were examined, amplification of fragment 1 showed a smaller band in addition to the normal PCR product (fig 1). Sequencing of the smaller band disclosed the removal of Ala60-Lys148, encoded by exon 2, which was consistent with skipping of this exon in lymphocytes. This previously undescribed alternatively spliced form of *DYT1* transcripts resulting in loss of frame was also observed in three normal controls.
### Table 1: Clinical, familial, and molecular data of the 35 index cases with early onset PTD tested for mutations in the DYT1 gene

<table>
<thead>
<tr>
<th>Patient (sex)</th>
<th>Age at onset</th>
<th>Site of onset</th>
<th>Age at generalisation</th>
<th>AGAG</th>
<th>Family history</th>
<th>Clinical features in affected relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Limb onset generalised dystonia without spread to cervicocranial muscles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (F)</td>
<td>7 y 6 mth</td>
<td>L. foot</td>
<td>9 y 3 mth</td>
<td>+</td>
<td>Yes</td>
<td>Mother: mild generalised dystonia</td>
</tr>
<tr>
<td>2 (F)</td>
<td>6 y 6 mth</td>
<td>R. hand</td>
<td>9 y 4 mth</td>
<td>+</td>
<td>Yes</td>
<td>PGF: writer's cramp, 2 unaffected</td>
</tr>
<tr>
<td>3 (M)</td>
<td>7 y</td>
<td>R. hand</td>
<td>7 y 7 mth</td>
<td>+</td>
<td>Yes</td>
<td>Father: spasmatic torticollis, 3 unaffected</td>
</tr>
<tr>
<td>4 (M)</td>
<td>15 y</td>
<td>Arm</td>
<td>?</td>
<td>+</td>
<td>Yes</td>
<td>4 generalised, 2 writer's cramp, 5 unaffected</td>
</tr>
<tr>
<td>5 (M)</td>
<td>12 y</td>
<td>R. hand</td>
<td>17 y</td>
<td>+</td>
<td>Yes</td>
<td>Mother: generalised dystonia</td>
</tr>
<tr>
<td>6 (F)</td>
<td>9 y</td>
<td>Arm</td>
<td>30 y</td>
<td>+</td>
<td>Yes</td>
<td>Cousin: multifocal dystonia</td>
</tr>
<tr>
<td>7 (M)</td>
<td>7 y</td>
<td>L. hand</td>
<td>11 y 8 mth</td>
<td>+</td>
<td>Yes</td>
<td>Father: blepharoospasm</td>
</tr>
<tr>
<td>8 (F)</td>
<td>9 y 8 mth</td>
<td>R. hand</td>
<td>11 y 8 mth</td>
<td>+</td>
<td>Yes</td>
<td>Brother: focal dystonia; MGF: writer's cramp, 1 unaffected</td>
</tr>
<tr>
<td>9 (M)</td>
<td>9 y</td>
<td>R. hand</td>
<td>10 y 6 mth</td>
<td>+</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>10 (F)</td>
<td>11 y 6 mth</td>
<td>L. foot</td>
<td>12 y 7 mth</td>
<td>+</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>11 (M)</td>
<td>7 y 1 mth</td>
<td>R. foot</td>
<td>9 y</td>
<td>+</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>12 (M)</td>
<td>7 y 9 mth</td>
<td>L. foot</td>
<td>10 y</td>
<td>+</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>Limb onset generalised dystonia with spread to cervicocranial muscles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 (M)</td>
<td>7 y 11 mth</td>
<td>R. hand</td>
<td>10 y 4 mth</td>
<td>+</td>
<td>Yes</td>
<td>Father: writer's cramp</td>
</tr>
<tr>
<td>14 (F)</td>
<td>6 y</td>
<td>L. foot</td>
<td>8 y</td>
<td>+</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>15* (M)</td>
<td>1 y 1 mth</td>
<td>R. hand</td>
<td>1 y 6 mth</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>16* (F)</td>
<td>18 mth</td>
<td>L. foot</td>
<td>3 y 6 mth</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>17* (F)</td>
<td>2 y</td>
<td>L. hand</td>
<td>5 y</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>18* (F)</td>
<td>2 y 7 mth</td>
<td>Arm</td>
<td>2 y 9 mth</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>19* (F)</td>
<td>3 y</td>
<td>R. foot</td>
<td>3 y 6 mth</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>20* (F)</td>
<td>3 y 6 mth</td>
<td>L. foot</td>
<td>8 y</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>21* (F)</td>
<td>5 y</td>
<td>R. hand</td>
<td>5 y 6 mth</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>22* (F)</td>
<td>8 y</td>
<td>L. foot</td>
<td>14 y</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>23* (F)</td>
<td>13 y</td>
<td>R. hand</td>
<td>14 y 6 mth</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>24* (M)</td>
<td>10 y</td>
<td>L. foot</td>
<td>15 y</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>25 (F)</td>
<td>4 y</td>
<td>F. foot</td>
<td>10 y</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>26 (M)</td>
<td>9 y</td>
<td>Leg</td>
<td>-</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>27 (M)</td>
<td>12 y 6 mth</td>
<td>Arm</td>
<td>14 y</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>28 (F)</td>
<td>12 y 6 mth</td>
<td>Arm</td>
<td>20 y</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>29 (F)</td>
<td>3 y 4 mth</td>
<td>Leg</td>
<td>13 y</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>Generalised dystonia with other affected sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30* (F)</td>
<td>2 y</td>
<td>Trunk, face</td>
<td>2 y 6 mth</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>31* (M)</td>
<td>13 y</td>
<td>Neck, trunk</td>
<td>18 y</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>32* (M)</td>
<td>22 y</td>
<td>Neck, trunk</td>
<td>22 y</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>33* (F)</td>
<td>17 y</td>
<td>Dysarthria</td>
<td>?</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>Focal dystonia (writer's cramp)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34* (M)</td>
<td>2 y 6 mth</td>
<td>R. hand</td>
<td>- (aged 11 y)</td>
<td>-</td>
<td>Yes</td>
<td>Sister: transient dystonia at 3 years</td>
</tr>
<tr>
<td>35* (M)</td>
<td>7 y</td>
<td>R. hand</td>
<td>- (aged 9 y)</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

PGF: paternal grandfather, MGF: maternal grandfather, *patients analysed for additional mutations by transcript analysis, ?: unknown.
proper folding of secreted and/or membrane
serves as a molecular chaperone assisting in the
nuclear envelope suggests that this protein
branes of the endoplasmic reticulum and
cation of torsinA as a lumenally associated
phisms (288C/T and 688G/C) in the coding
transcripts, 2: normal sized RT-PCR fragment containing
detection of alternative exon 2 skipping in DYT1
molecular weight marker (1 kb ladder, Gibco BRL), 1:
accounting for the reduced size of the mutant fragment. M:
showed that the 266 bp exon 2 was missing, thereby fully
accounting for the reduced size of the mutant fragment. M:
molecular weight marker (1 kb ladder, Gibco BRL), 1:
detection of alternative exon 2 skipping in DYT1
transcripts, 2: normal sized RT-PCR fragment containing
exons 1-3.

Moreover, two previously reported polymor-
phisms (288C/T and 688G/C) in the coding
sequence were detected in this group of
patients.

Discussion
Our data agree with a previous report by
Ozelius et al., who failed to identify any new
change in the DYT1 (TOR1A) gene, and its
human homologue TOR1B, in 17 patients with
features typical of early onset dystonia, by using a
DNA based approach. In the present study,
an RNA based strategy was used to allow
detection of partial gene deletions (or duplica-
tions) and inclusions of intronic sequences, in
addition to amino acids substitutions. Even
though it remains possible that some mutations in
untranslated regions could have been
missed, these results may indicate that the
ΔGAG deletion is the only DYT1 mutation
responsible for early onset PTD.

The predominance of this mutation may
reflect an increased frequency of this sequence
alteration owing to genetic instability in an
imperfect tandem of a 24 bp repeat in the
region of the deletion. Also, it has been
hypothesised that the inability to identify addi-
tional mutations in the DYT1 gene indicates
that the new torsinA protein conformation
resulting from the GAG deletion (loss of a
glutamic acid residue in the carboxy terminal) is
functionally unique. TorsinA bears low but
significant homology to the Hsp100/Clp family
of ATPase chaperones. The recent identifi-
cation of torsinA as a lumenally associated
glycoprotein localised to intracellular mem-
branes of the endoplasmic reticulum and
nuclear envelope suggests that this protein
serves as a molecular chaperone assisting in the
proper folding of secreted and/or membrane
proteins. Several molecular mechanisms have
been proposed. The mutant form of torsinA
may (1) sequester torsinA in inactive com-
plexes, thus acting as a dominant negative, (2)
behave as a constitutively active torsinA, thus
acting as a dominant positive, and/or (3)
possess novel functionality distinct from the
biological roles of torsinA. The
identification of a single mutation on
affected chromosomes responsible for almost
all cases of typical early onset dystonia is
remarkable. The GAG deletion would be one
of the rare examples of the same recurrent and
unique mutation causing a dominantly inher-
ited condition. Other examples include hy-
pokalaemic periodic paralysis, achon-
droplasia, hypertrophic cardiomyopathy, and
the triplet repeat disorders. In all these cases, it
appears that the same mutation occurs repeat-
edly as independent events, whereas other
mutations in the same gene cause a different
syndrome, have no phenotype, or are incompat-
ible with life.

To date, the DYT1 gene is the only primary
PTD gene identified for which a direct DNA
based genetic test is available. Our results con-
firm a genotype/phenotype correlation in early
onset PTD since the 14 DYT1 positive index
cases displayed a characteristic phenotype. The
phenotype is marked by onset in a limb, usually
before 24 years of age, frequently spreading to
other limbs, occasionally to the neck, and rarely
to cranial muscles. However, a variable
progression of dystonia was observed in familial
cases, and atypical features such as blepharospasm
may be the only sign. It is still unclear
whether these phenotypes represent variable
expression of the GAG mutation or the effect
of modifying gene or environmental factors. It
has been suggested that the GAG deletion in
the DYT1 gene would increase a person’s
susceptibility to a “second hit” brought on by
either genetic or environmental factors. This
observation extends the clinical spectrum of
DYT1 associated dystonia and shows the
significance of molecular testing in establishing
the clinical diagnosis of hereditary dystonic
syndromes. As previously stated, DYT1 diag-
nostic testing in subjects with atypical clinical
features or late onset may be warranted in those
having a positive family history of early onset
PTD.

In accordance with a previous report, we
found no deletion carriers among patients with
onset in early childhood (younger than 5
years). The delay of generalisation tends to be
shorter (within a few months) in this group of
patients compared to DYT1 patients. Accord-
ing to Fahn et al., age is the most important
single factor related to the prognosis of
idiopathic dystonia. The younger the age at
onset, the more likely the dystonia will become
severe and also spread to multiple parts of the
body. In conclusion, comprehensive screening
of the whole DYT1 coding sequence in patients
with early onset PTD argues for differences in
disease causing loci of early onset autosomal
dominant dystonia, with additional responsible
gene(s) yet to be identified. PTD is a clinically
and genetically heterogeneous group of move-
ment disorders. Three other PTD loci have
been mapped to date, each associated with a
relatively well defined phenotype, although
there is substantial phenotypic overlap in indi-
vidual cases. The DYT7 locus on chromosome
18p is associated with adult onset focal cervical
dystonia, whereas the DYT6 locus on chro-
mosome 8 is associated with a mixed pheno-
type (the symptoms range from purely focal to

Figure 1 Total RNA from peripheral blood lymphocytes
was reverse transcribed and PCR amplified (RT-PCR)
using primers located upstream of exon 1 (forward) and in
exon 3 (reverse). The products were analysed on a 2.5%
agarose gel. The normal product expected for fragment 1 is
578 bp; a shorter fragment (312 bp) was observed in five
patients and in three normal controls in addition to the
normal fragment. Sequencing of the 312 bp fragment
showed that the 266 bp exon 2 was missing, thereby fully
accounting for the reduced size of the mutant fragment. M:
molecular weight marker (1 kb ladder, Gibco BRL), 1:
detection of alternative exon 2 skipping in DYT1
transcripts, 2: normal sized RT-PCR fragment containing
exons 1-3.
generalised dystonia with variable age of onset. Recently, a novel PTD locus, DYT13 on chromosome 1p, has been characterised in a large Italian family with prominent cranio-cervical and upper limb involvement and mild severity. Other genes involved in the aetiology of dystonia, particularly in early onset generalised PTD, remain to be characterised.

We thank all patients and family members for participation in this study. We also wish to thank Professor I, Ozellus for providing DNA from a DYT1 deletion carrier, N Vaysseire and I Cas (URMAE, Hôpital Gui de Chauliac, Montpellier) for collecting clinical data, Dr P Castelnau and Professor P Lévard (Hôpital R Débré, Paris), Dr O Boespflug-Tanguy (CHU Clermont-Ferrand) for sending blood samples, and all our neurological colleagues for referring patients. This work was partly supported by the Research Group on Movement Disorders (URMAE, Hôpital Gui de Chauliac, Montpellier, France).


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