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Research report

# TorsinA expression is detectable in human infants as young as 4 weeks old

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**Abstract**

Familial, early onset, generalized torsion dystonia is the most common and severe primary dystonia. The majority of cases are caused by a 3-bp deletion (GAG) in the coding region of the DYT1 (TOR1A) gene. The cellular and regional distribution of torsinA protein and its message has been described previously in several regions of normal adult human and rodent brain. This study examines the expression of torsinA in the developing human brain of fetuses, infants and children up to 7 years of age in four selected brain regions. Expression of torsinA protein was detectable beginning at 4 to 8 weeks of age postnatally in the cerebellum (Purkinje cells), substantia nigra (dopaminergic neurons), hippocampus and basal ganglia. Prominent torsinA immunoreactivity was not seen before 6 weeks of age postnatally, a period associated with synaptic remodeling, process elimination and the beginning of myelination. Our results indicate that torsinA protein expression is temporally and spatially regulated and is present in all brain regions studied by the age of 2 months on into adulthood.


*Theme:* Neurologic disorders
*Topic:* Dystonia

*Keywords:* TorsinA; Brain; Children; Neurons; Development; Dopaminergic

## 1. Introduction

Recently, a genetic defect associated with generalized, early onset torsion dystonia, the most common form of hereditary dystonia, has been identified [26]. A 3-bp (GAG) deletion in the coding region of the DYT1 gene, which is located on chromosome 9q34 [25,26], accounts for the majority of cases with this form of dystonia, especially in individuals of Ashkenazi Jewish descent [17]. In this autosomal dominant condition with incomplete (30–40%) penetrance, symptoms usually begin in childhood and affect the limbs first, with subsequent involvement of multiple body parts [6]. The DYT1 gene, which encodes the protein, torsinA, is a member of a gene family with three homologous members in humans, torsinB, torp2 (torp2a) and torp3 (torp3a) [5,26]. The cellular function of torsinA, which is expressed in multiple tissues and species, remains unknown [5].

Recent in-situ hybridization and immunohistochemistry studies demonstrated high expression of torsinA mRNA in several regions of the adult human brain, including the dopaminergic neurons of the substantia nigra pars compacta, granule and pyramidal cells of the hippocampal formation, Purkinje and dentate nucleus neurons of the cerebellum and cholinergic neurons of the neostriatum [1,3,27,30]. Similar staining patterns at the light microscopic level were also detected in subjects with DYT1 dystonia, suggesting cellular dysfunction in the absence of cell loss or significant torsinA protein loss [27].

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To extend these observations, we have studied the temporal and spatial expression of torsinA in four selected regions of the developing human brain (hippocampus, basal ganglia, midbrain including substantia nigra and cerebellum) by immunohistochemistry using a new monoclonal antibody D-M2A8, specific to torsinA [13].

## 2. Methods

### 2.1. Human brain tissue

Paraffin embedded brain blocks (hippocampus, basal ganglia, midbrain including substantia nigra and cerebellum) from 15 control subjects with an age range of 20 weeks of gestation to 7 years and two adults were obtained from the Department of Neuropathology, University of Göttingen, Germany (Table 1). In addition, fresh frozen brain tissue from one adult control and three infants was obtained for Western blot studies. All subjects had no history of neurological disease and no abnormalities on neuropathological examination. DNA was extracted from brain tissue to evaluate the status of the GAG deletion in DYT1. The postmortem interval before fixation in buffered formalin was less than 24 h in all cases.

Table 1
Normal human postmortem tissue

| Subjects | Age at death/sex | Cause of death |
|---|---|---|
| 1[a] | 20 weeks of gestation/F[b] | Spontaneous abortion |
| 2 | 23 weeks of gestation/M[c] | Spontaneous abortion |
| 3 | 1 mo[d]/M | Suspected SIDS[e] |
| 4 | 6 weeks/M | Suspected SIDS |
| 5 | 2 mo/F | Pneumonia |
| 6 | 2 mo/M | Suspected SIDS |
| 7 | 7 mo/M | Congenital heart disease |
| 8 | 8 mo/M | Pneumonia |
| 9 | 8 mo/F | Congenital heart disease |
| 10 | 2.5 y[f]/M | Drowning |
| 11 | 2.5 y/F | Adverse drug reaction |
| 12 | 3 y/M | Carbon monoxide poisoning |
| 13 | 4 y/M | Laryngospasm |
| 14 | 4 y/M | Carbon monoxide poisoning |
| 15 | 7 y/M | Acute heart failure |
| 16 | 33 y/M | Septic shock |
| 17 | 62 y/M | Gastrointestinal bleeding |
| 18[g] | 31 y/M | Not known |
| 19 | 62 y/M | Not known |
| 20 | 1 mo/F | Suspected SIDS |
| 21 | 3 mo/M | Suspected SIDS |
| 22 | 9 mo/M | Suspected SIDS |

Subjects 1–17: used for immunohistochemistry.
[a] Subjects 1–17 were genotyped and were found to be negative for the GAG deletion in the DYT1 gene.
[b] F—female.
[c] M—male.
[d] mo—month.
[e] SIDS—sudden infant death syndrome.
[f] y—years.
[g] 18–22: two adults and three infants used for Western blot studies.

### 2.2. Genotyping

DNA was extracted from paraffin embedded frontal lobe cortex, as previously described [19]. The 3-bp GAG deletion in the DYT1 gene was assayed by polymerase chain reaction (PCR) with [$\alpha^{32}$P]-dGTP (Perkin-Elmer Life Sciences, Boston, MA). Primer sequences were as follows: GAGS: 5′-CCCGAGGCTATGAAATTG-3′ and GAGAS: 5′-TCTGAGAAAACTCTC TCCTC-3′ yielding fragments that spanned the GAG deletion. PCR products were resolved by electrophoresis in a denaturing 6% monomer sequaGEL-6 (National Diagnostics, Atlanta, GA) polyacrylamide gel and visualized by autoradiography. By this method, samples from affected individuals heterozygous for a GAG deleted allele and normal allele display two distinct bands of 85 and 88-bp, whereas normal homozygotes show a single 88-bp band [27].

### 2.3. Western blot

Samples for brain homogenates were taken from frontal lobe and homogenized in RIPA buffer [150 mM NaCl, 50 mM Tris pH 8.0, 1% NP40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate (SDS)] by sonication (550 Sonic Dismembrator, Fisher Scientific, Dortmund, Germany) at a setting of 3.5 for 30 s. Homogenates were allowed to sit on ice for 30 min then centrifuged at 16,000 × *g* for 30 min. The protein concentration in the supernatant was determined using the bicinchoninic acid (BCA) assay. After adding SDS-PAGE sample buffer, the samples were heated for 10 min in a boiling water bath. Equal amounts of protein per well were loaded on 12.5% polyacrylamide gels. Following electrophoresis, the proteins were transferred from the gel to a nitrocellulose membrane (Schleicher and Schuell, Kassel, Germany) using the semi-dry method. The membranes were blocked and incubated with the torsinA antibody D-M2A8 [13] at a final dilution of 1:5000. Finally, the blots were developed with a horseradish peroxidase (HRP)-coupled goat-anti-mouse antibody (1:1000, Dako-Envision, Glostrup, Denmark) using chemiluminescence detection on Hyperfilm (Amersham Biosciences, Freiburg, Germany). Anti-tubulin (Sigma, St. Louis, MO) was used at a final dilution of 1:10,000.

### 2.4. Immunohistochemistry

Two-micron sections were cut and mounted on precleaned sialinized slides (Sigma). The sections were deparaffinized and rehydrated using xylene and graded changes of ethanol. Three affinity-purified antibodies to torsinA were used: a rabbit polyclonal antibody, TAB1 (1:20), a mouse monoclonal antibody, D-MG10 (1:1000) [12], and a new mouse monoclonal antibody, D-M2A8 (1:50) [13]. TAB1