Defects in the striatal neuropeptide Y system in X-linked dystonia-parkinsonism

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Neuropeptide Y is a novel bioactive substance that plays a role in the modulation of neurogenesis and neurotransmitter release, and thereby exerts a protective influence against neurodegeneration. Using a sensitive immunohistochemical method with a tyramide signal amplification protocol, we performed a post-mortem analysis to determine the striatal localization profile of neuropeptide Y in neurologically normal individuals and in patients with X-linked dystonia-parkinsonism, a major representative of the neurodegenerative diseases that primarily involve the striatum. All of the patients examined were genetically verified as having X-linked dystonia-parkinsonism. In normal individuals, we found a scattered distribution of neuropeptide Y-positive neurons and numerous nerve fibres labelled for neuropeptide Y in the striatum. Of particular interest was a differential localization of neuropeptide Y immunoreactivity in the striatal compartments, with a heightened density of neuropeptide Y labelling in the matrix compartment relative to the striosomes. In patients with X-linked dystonia-parkinsonism, we found a significant decrease in the number of neuropeptide Y-positive cells accompanied by a marked loss of their nerve fibres in the caudate nucleus and putamen. The patients with X-linked dystonia-parkinsonism also showed a lack of neuropeptide Y labelling in the subventricular zone, where a marked loss of progenitor cells that express proliferating cell nuclear antigen was found. Our results indicate a neostriatal defect of the neuropeptide Y system in patients with X-linked dystonia-parkinsonism, suggesting its possible implication in the mechanism by which a progressive loss of striatal neurons occurs in X-linked dystonia-parkinsonism.

Keywords: neuropeptide Y; X-linked dystonia-parkinsonism; striatum; neurodegeneration; neurogenesis

Abbreviation: MEnk = Met-enkephalin
Introduction

X-linked dystonia-parkinsonism (MIM314250), also known as DYT3 dystonia or ‘Lubag’ disease, was first described as an endemic disease on the island of Panay in the Philippines (Lee et al., 1976). X-linked dystonia-parkinsonism is now classified as one of the primary monogenic dystonias (Müller, 2009), and this adult-onset movement disorder is clinically characterized by progressive and severe dystonia followed by overt parkinsonism in the later years of life (Lee et al., 2002). Genetic molecular analyses have shown that X-linked dystonia-parkinsonism is a transcriptional dysregulation syndrome with impaired expression of the TAF1 (TATA box-binding protein associated factor 1) gene (Nolte et al., 2003), an essential component of the transcription machinery (Wassarman and Sauer, 2001). We also found a reduced expression of the neuron-specific isoform of the TAF1 gene in patients with X-linked dystonia-parkinsonism (Makino et al., 2007). The major neuropathology of X-linked dystonia-parkinsonism is a progressive neuronal loss that occurs in the striatum (Lee et al., 2002; Goto et al., 2005); however, the underlying pathomechanism for striatal neurodegeneration that causes dystonia symptoms in X-linked dystonia-parkinsonism remains to be elucidated.

Neuropeptide Y, a 36-amino acid peptide, is abundantly expressed in multiple brain regions (Tatemoto et al., 1982), and neurons expressing neuropeptide Y in one region have no anatomical or functional connections with neuropeptide Y neurons in other brain regions (van den Pol, 2012). In the striatum, neuropeptide Y is frequently expressed in a subclass of GABAergic interneurons, which contains somatostatin and nitric oxide synthase (Tepper et al., 2010). Neuropeptide Y is a powerful neuroactive substance that has been implicated in a variety of brain disorders (Xapelli et al., 2000; Benarroch, 2009; Decressac and Barker, 2012). Of particular interest is that neuropeptide Y plays an important role in neuroprotection against excitotoxicity (Xapelli et al., 2006; Decressac and Barker 2012; Malva et al., 2012), and in the modulation of neurogenesis (Hansel et al., 2001; Curtis et al., 2007; Agasse et al., 2008; Stanić et al., 2008; Decressac and Barker, 2012; Malva et al., 2012) and glutamate and dopamine neurotransmissions (Xapelli et al., 2006; Decressac and Barker, 2012; van den Pol, 2012). In this study, we have demonstrated a neostriatal defect of the neuropeptide Y system in patients with X-linked dystonia-parkinsonism, in addition to the characteristic localization profile of neuropeptide Y in the human striatum. Our results have important implications for understanding the mechanisms by which a progressive striatal neurodegeneration associated with dystonia genesis occurs in X-linked dystonia-parkinsonism.

Subjects and methods

Autopsied human brains and tissue preparation

All procedures involving post-mortem human brain tissues were approved by the Ethical Review Committee of the University of Tokushima. Brain tissues were obtained at autopsy from four male Filipino patients with X-linked dystonia-parkinsonism (mean age ± SEM, 50.3 ± 7.7), and from four neurologically normal individuals (57.5 ± 9.0). The clinical characteristics of the patients with X-linked dystonia-parkinsonism are summarized in Table 1. The brain tissues were fixed in 10% neutral formalin and embedded in paraffin; 4-µm thick tissue sections were then cut with a microtome and mounted onto Matsunami Adhesive Silane (MAS)-coated glass slides (Matsunami Glass). Conventional neuropathological examinations were performed with Nissl (Klüver-Barrera), and haematoxylin-eosin staining.

Genetic testing

The insertion mutation of SVA (short interspersed nuclear element, VNTR, and Alu composite) retrotransposon in intron 32 of TAF1 has been considered to be causative for X-linked dystonia-parkinsonism (Makino et al., 2007). Previously termed X-linked dystonia-parkinsonism disease-specific changes 3 (DSC3) within the X-linked dystonia-parkinsonism locus is in strong linkage disequilibrium with the retrotransposon and is regarded as allele associated with X-linked dystonia-parkinsonism (Nolte et al., 2003).

Genomic DNA was extracted from formalin-fixed paraffin-embedded brain tissue as described elsewhere (Perizzolo et al., 2012). As the SVA retrotransposon of 2627 base pairs in length could not be amplified using long range PCR, DSC3 was investigated instead, according to the method reported previously (Nolte et al., 2003). In all samples of the four patients with X-linked dystonia-parkinsonism examined, the mutated allele (C > T transition) presumably in hemizygous state, was confirmed by sequencing (Fig. 1).

Immunohistochemistry

After routine deparaffinization, rehydration and blocking of endogenous peroxidase activity, all sections to be used for immunostaining were processed for microwave-enhanced antigen retrieval. Slide-mounted sections immersed in 0.01 M sodium citrate buffer (pH 6.0) were placed in a 700 W microwave oven for 15 min at maximum power. Sections were blocked in PBS (pH 7.2) containing 3% bovine serum albumin for 60 min at room temperature and then incubated with primary antibodies for 18 h at room temperature in PBS containing 3% bovine serum albumin. Rabbit polyclonal antibodies for neuropeptide Y (Affiniti, 1:4000), and Met-enkephalin (MEnk; Millipore, 1:50 000) were used as primary antibodies. After several rinses in PBS, the sections were incubated with biotinylated secondary antibodies for rabbit IgG (Vector Laboratories, 1:500) followed by the avidin-biotin peroxidase complex (ABC) staining according to the manufacturer’s instructions (Vectastain® Elite ABC kit, Vector). The sections were then processed for tyramide signal amplification using the TSA™ Biotin System (Perkin Elmer). Briefly, the sections were incubated in biotinyl tyramide amplification reagent for 30 min at room temperature, followed by the ABC complex (Vector). The bound peroxidase was visualized by incubating the sections with a solution containing 3,3′-diaminobenzidine and H2O2.

For double immunofluorescence staining, dual antigen detection with the tyramide signal amplification system was carried out according to the method reported previously (Okita et al., 2012). Briefly, sections were first incubated in PBS containing 3% bovine serum albumin and anti-neuropeptide Y antibodies (Affiniti; 1:4000) for 18 h at room temperature. The bound primary antibodies were detected by the Histofine Simple Stain Kit (Nichirei) and the tyramide signal amplification system with Cyanine 3 (Perkin Elmer LAS). To remove the
bound antibodies, the stained sections were then incubated in 0.1 M glycine-HCl (pH 2.2) at room temperature for 30 min. After incubation in PBS for 1 h, the sections were incubated for 18 h at room temperature in PBS containing 3% bovine serum albumin and antibodies for MEnk (Millipore, 1:50000) or calcineurin (1:1000) (Goto et al., 2005).

The bound antibodies were detected by the Histofine Simple Stain Kit (Nichirei) and the tyramide signal amplification system with Fluorescein (Perkin Elmer). Single staining for proliferating cell nuclear antigen was also carried out using the tyramide signal amplification system with Cyanine 3 (Perkin Elmer LAS). Anti-proliferating cell nuclear antigen

<table>
<thead>
<tr>
<th>XDP</th>
<th>Age/Sex</th>
<th>Duration of illness (years)</th>
<th>Cause of death</th>
<th>Initial symptoms</th>
<th>Symptoms at death</th>
<th>Number of years to generalization</th>
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<td>54 years/male</td>
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<td>Pneumonia</td>
<td>Writer’s cramp</td>
<td>Generalized dystonia</td>
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<td>Patient 2</td>
<td>46 years/male</td>
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<td>Pneumonia</td>
<td>Tongue protrusion</td>
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<tr>
<td>Patient 3</td>
<td>42 years/male</td>
<td>6</td>
<td>Suicide</td>
<td>Facial grimacing</td>
<td>Generalized dystonia</td>
<td>6</td>
</tr>
<tr>
<td>Patient 4</td>
<td>59 years/male</td>
<td>3</td>
<td>Suicide</td>
<td>Mouth opening</td>
<td>Generalized dystonia</td>
<td>3</td>
</tr>
</tbody>
</table>

Normal control subjects
Case 1 49 years/male Pneumonia
Case 2 52 years/male Heart failure
Case 3 60 years/male Myocardial infarction
Case 4 69 years/male Pneumonia

XDP = X-linked dystonia-parkinsonism.

**Table 1 Subject descriptive data**

**Figure 1** Genomic sequence chromatograms of the disease-specific changes 3 (DSC3). (A–D) The C>T substitution was demonstrated in the four human samples investigated in this study. (E) The C>T substitution was revealed in a disease control, in which SVA retrotransposon in intron 32 of the TAF1 gene was demonstrated using long range PCR (data not shown). (F) Heterozygous genotype (C/T) was demonstrated in an obligate female carrier. (G) Hemizygous for a wild allele C was shown in a healthy control. XDP = X-linked dystonia-parkinsonism.
antibodies (Santa-Cruz, 1:5000) were used as primary antibodies, according to the method described above.

**Digital microscopic images and morphometry**

Digital microscopic images were captured using an Olympus BX51 microscope, imported into Adobe Photoshop CS4, and processed digitally for adjustments of contrast, brightness, and colour balance. Morphometric analyses were carried out in a blind manner. To determine the cell density, we counted the numbers of neuropeptide Y-positive cells with clear nuclei in a 1 × 1 mm field in the striatum. We examined five fields selected randomly in each of the caudate nucleus, putamen, or nucleus accumbens from each patient with X-linked dystonia-parkinsonism (n = 4) and normal control subjects (n = 4). The percentage of the population of striatal cells labelled for neuropeptide Y (n = 1000) was also calculated in striatal sections (n = 3) that were doubly stained for neuropeptide Y and MEnk in each normal individual (n = 4). The subfields of the striosomes and matrix compartment were determined as follows. The striosome was divided into the core and annular zones, and the matrix was divided into the area near (<100 μm in distance) to the striosome-matrix border (border zone) and the remaining area (non-border zone). To determine the number of proliferating cell nuclear antigen-positive cells in the subependymal zone of the subventricular zone, we counted immunoreactive cells in a sample area (1 mm in length) from each patient with X-linked dystonia-parkinsonism (n = 4) and normal control subjects (n = 4).

**Statistical analysis**

All experimental values are expressed as means ± SEM. Statistical significance was evaluated by Mann-Whitney U-test. A P-value < 0.05 was considered statistically significant.

**Results**

**Striatal localization of neuropeptide Y in neurologically normal individuals**

Using highly sensitive tyramide signal amplification immunohistochemical techniques, we successfully detected neuropeptide Y immunoreactivity in the formalin-fixed paraffin-embedded human autopsied tissues (Fig. 2). Strong neuropeptide Y labelling was found in the striatum, which showed a scattered distribution

![Figure 2](image-url)
of neuropeptide Y-positive neuronal cell bodies surrounded by extensive nerve fibre staining (Fig. 2). Notably, at low-power magnifications, neuropeptide Y labelling was observed to be differentially localized in the striosome and matrix compartments of the striatum (Fig. 3). Compared with the striosomes, the matrix compartment was more strongly stained for neuropeptide Y, as determined using the serial sections stained for neuropeptide Y (Fig. 3A) and MEnk (Fig. 3B), a marker for striosomes in autopsied human brains (Goto et al., 1990). Double immunofluorescence staining with the tyramide signal amplification protocols also showed that neuropeptide Y immunoreactivity was poor in striosomes that exhibited high MEnk labelling (Fig. 3C and D). The margins of the neuropeptide Y-poor zones closely corresponded with the outer margins of the striosome annular zones, also known as ‘annular compartments’ (Holt et al., 1997), which were identified by MEnk staining (Fig. 3E). Notably, fluorescent microscopy showed that at low-power magnifications, the heightened neuropeptide Y labelling density in the matrix became obscured when using a short exposure time (Fig. 4A–C). This was in parallel with the findings obtained under high magnification (Fig. 4D and E), in which the nerve fibre staining was almost diminished, but cell body staining was still evident. Thus, it is likely that the enrichment of neuropeptide Y labelling in the matrix was largely due to a differential density of neuropeptide Y-positive nerve fibres between the striosome and matrix compartments.

On striatal sections that were doubly stained for MEnk and neuropeptide Y (Fig. 5), we determined the distributional profile of neuronal cell bodies stained for neuropeptide Y in the subfields of the striosome-matrix systems. As shown in Fig. 6A, the striosome was divided into core and annular zones, and the matrix was divided into the area near (<100 μm in distance) to the striosome-matrix border (border zone) and the remaining area (non-border zone). The population percentage study (Fig. 6B) revealed that 6.2 ± 1.3%, 18.9 ± 5.4%, 25.9 ± 7.6%, and 47.3 ± 8.2% of all striatal neuropeptide Y neurons were found in the core, annular, border, and non-border zones, respectively. This finding indicates that ~75% of the striatal neuropeptide Y neurons were located in the matrix compartment, while ~50% of the striatal neuropeptide Y neurons were located near or within the striosomes. When taken into account with the fact that striosomes make up 10–20% of the volume of the striatum (Graybiel, 1990), our results suggest that neuropeptide Y-positive cell bodies might be more highly concentrated in the striatal area near or within the striosomes than in the remaining matrix compartment.

**Figure 3** The differential distribution of neuropeptide Y in the striosome and matrix compartments of normal individuals. (A and B) Direct reversed images of the serial striatal sections immunostained for (A) neuropeptide Y (NPY) and (B) MEnk, a marker of striosomes. Asterisks indicate a corresponding striosome that is poor in neuropeptide Y but enriched in MEnk. (C and D) Photomicrographs of the striatum doubly-stained for neuropeptide Y (C) and MEnk (D). Asterisks indicate the striosomes poor in neuropeptide Y in (C). Arrows indicate the striosomes enriched in MEnk (D). (E) A schematic representation of the matrix enriched in neuropeptide Y (red) and the striosome enriched in MEnk (green). Scale bars: A and B = 4 mm; C and D = 1 mm. CN = caudate nucleus; PUT = putamen; D = dorsal; M = medial; V = ventral; L = lateral.
Striatal pathology of patients with X-linked dystonia-parkinsonism with generalized dystonia

We then reappraised the striatal pathology of the patients with X-linked dystonia-parkinsonism with generalized dystonia. All four patients with X-linked dystonia-parkinsonism enrolled in this study were male, and the mean age at disease onset was 41.3 years (range 34–56 years; Table 1). The mean duration of illness was 9 years (range 3–14 years); two patients committed suicide. All patients initially manifested focal dystonias and within 6 years developed generalized dystonia (Table 1).

A conventional pathological analysis revealed definite atrophy of the neostriatum (i.e. the caudate nucleus and putamen), with a sparing of the nucleus accumbens, in all patients with X-linked dystonia-parkinsonism (Fig. 7B), compared with normal individuals (Fig. 7A). Neostriatal lesions manifested with a striking mosaic appearance of neurodegeneration resulting from islands of normal-looking striatum (X-linked dystonia-parkinsonism patches) interspersed among sharply demarcated astrogliosis areas (Waters et al., 1993; Goto et al., 2005). These X-linked dystonia-parkinsonism patches were appreciated best (Fig. 7C and D) with immunostaining for calcineurin, a neurochemical marker for medium spiny neurons in the striatum (Goto et al., 2005).

Disruption of the neuropeptide Y system in the neostriatum of patients with X-linked dystonia-parkinsonism

Representative reverse images of the striatal sections obtained from patients with X-linked dystonia-parkinsonism and stained for calcineurin and neuropeptide Y are shown in Fig. 8A and B, respectively. In patients with X-linked dystonia-parkinsonism, loss of neuropeptide Y labelling was markedly seen in the caudate nucleus and putamen, whereas it was not apparent in the nucleus accumbens. Microscopically, the X-linked dystonia-parkinsonism patches stained for neuropeptide Y were identifiable (Fig. 8D), but they were less evident than those stained for calcineurin (Fig. 8C). Neuropeptide Y-positive cells were seen in both the X-linked dystonia-parkinsonism patches (Fig. 8E) and the inter-patch area (Fig. 8F). The population percentage study (Fig. 8G) revealed that neuropeptide Y-positive cells were almost evenly distributed in the X-linked dystonia-parkinsonism patches and in the inter-patch area. We also found a decrease in the number of neuropeptide Y-positive neurons accompanied by a severe loss of their neuropeptide Y-labelled nerve fibres in both the caudate nucleus (Fig. 9B) and putamen (Fig. 9E), but not in the nucleus accumbens (Fig. 9H), in patients with X-linked dystonia-parkinsonism as compared with normal individuals (Fig. 9A, D and G). Cell density analysis confirmed this and revealed a statistically
significant reduction in the number of neuropeptide Y-positive cells in the caudate nucleus (Fig. 9C; \(P < 0.001\), Mann-Whitney U-test) and putamen (Fig. 9F; \(P < 0.001\), Mann-Whitney U-test) but not in the nucleus accumbens (Fig. 9I; \(P > 0.05\), Mann-Whitney U-test) in patients with X-linked dystonia-parkinsonism, as compared with normal control subjects.

Do patients with X-linked dystonia-parkinsonism have a deficit in subventricular zone neurogenesis?

Graded colour-converted images of the striatum stained for neuropeptide Y showed heightened neuropeptide Y labelling in the subventricular zone of normal individuals (Fig. 10A). In contrast, patients with X-linked dystonia-parkinsonism showed a lack of neuropeptide Y labelling in the subventricular zone (Fig. 10B). This was also evident in microscopic images (Fig. 10C and D). Further, cells positive for proliferating cell nuclear antigen, a marker for subventricular zone progenitor cells (Curtis et al., 2005), were abundantly found in normal control subjects (Fig. 10E and G), but they were markedly depleted in patients with X-linked dystonia-parkinsonism (Fig. 10F). Cell count analysis confirmed this and revealed a statistically significant reduction in the number of proliferating cell nuclear antigen-positive cells in the subependymal zone of patients with X-linked dystonia-parkinsonism (Fig. 10H; \(P < 0.001\), Mann-Whitney U-test), when compared

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**Figure 5** Localization of neuropeptide Y-positive cells in the striosome-matrix system of normal individuals. Double immunofluorescence staining for MEnk (A), and neuropeptide Y (B), with a merged image (C). Asterisks in (A) indicate striosomes. The region shown in the open dashed box in (C) is illustrated at higher magnification in (D), where neuropeptide Y-positive cell bodies are indicated by open circles. Scale bars = 500 \(\mu\)m. V = vessel.
with normal control subjects. Thus, it is likely that patients with X-linked dystonia-parkinsonism have a deficit in subventricular zone neurogenesis and that this deficit is associated with the loss of neuropeptide Y.

**Discussion**

In this study, we documented a novel finding regarding the distributional profile of neuropeptide Y in the human striatum. A sensitive tyramide signal amplification immunohistochemical technique allowed us to obtain subtle neuropeptide Y-staining signals in formalin-fixed paraffin-embedded autopsied human brain tissues. As in previous reports on primate and rodent brains (de Quindt and Emson, 1986; Smith and Parent, 1986; Caberlotto et al., 2000), our study showed that neuropeptide Y-containing neurons are scattered throughout the entire striatum in normal individuals. Of particular interest was the finding that neuropeptide Y immunoreactivity was differentially concentrated in the striosome-matrix systems, with higher density of neuropeptide Y labelling in the matrix compartment than in the striosomes. This novel finding may provide a new insight into understanding the striosome-matrix pathology that involves motor and behavioural brain dysfunctions (Graybiel et al., 2000; Graybiel, 2008; Goto et al., 2010; Crittenden and Graybiel, 2011).

So far, it has been postulated that in several striatal disorders such as Huntington’s disease and cerebral ischaemia, the loss of striatal neurons may relate to excitotoxicity caused by excessive levels of extracellular glutamate (Calabresi et al., 1998; Zeron et al., 2001; Heng et al., 2009). On the other hand, accumulating data have shown that neuropeptide Y potentially exerts a neuroprotective action against excitotoxicity by inhibiting glutamate release in the brain (Xapelli et al., 2006; Decressac and Barker, 2012). Thus, diminished neuropeptide Y expression could
contribute to excitotoxic injury that might occur in the X-linked dystonia-parkinsonism striatum. Given the present finding that the striosomes compartment has lower neuropeptide Y immunoreactivity relative to the matrix, we hypothesize that compared with the matrix compartment, the striosomes might be more susceptible to excitotoxicity. Indeed, a predominant loss of striatal medium spiny neurons in the striosome compartment has been shown in patients with Huntington’s disease (Hedreen and Folstein, 1995) and in a rodent model for Huntington’s disease (Lawhorn et al., 2008) or cerebral ischaemia (Burke and Baimbridge, 1993). Our hypothesis may be relevant to the striatal pathology of X-linked dystonia-parkinsonism, in which there is a preferential loss of striosomes with relative sparing of the matrix compartment (Goto et al., 2005). Moreover, similar to Huntington’s disease and cerebral ischaemia (Calabresi et al., 1998), X-linked dystonia-parkinsonism shows preferential loss of striatal medium spiny neurons whereas cholinergic interneurons are spared (Goto et al., 2005); this cell type-specific loss of neurons is a hallmark in the pathology of striatal excitotoxic lesions.

A growing body of evidence shows that impaired activity of the striosome compartment could participate in motor and behavioural brain disorders. Striosomes are thought to communicate with the matrix compartment through striatal interneurons that include cholinergic interneurons, GABAergic fast-spiking interneurons containing parvalbumin, and GABAergic low-threshold spiking interneurons containing neuropeptide Y, somatostatin and nitric oxide synthase. These interneurons often lie at the border between the striosome and matrix, and extend their dendrites across compartmental boundaries (for a review see Miura et al., 2008; Crittenden and Graybiel, 2011). Our results show that ~50% of neuropeptide Y-positive cell bodies were localized in the matrix area near to or within the striosomes. This notion may corroborate with the findings of a previous report on rodent brain (Kubota and Kawaguchi, 1993), supporting the general concept that neuropeptide Y-positive cells are one of the GABAergic interneurons that play a role in the inter-compartmental communication in the striatum (Miura et al., 2008; Crittenden and Graybiel, 2011).

![Figure 8](https://example.com/figure8.png) Striatal neuropeptide Y pathology in patients with X-linked dystonia-parkinsonism. (A and B) Direct reversed images of the striatal sections immunostained for calcineurin (CaN) (A) and neuropeptide Y (NPY) (B). (C and D) Photomicrographs of the X-linked dystonia-parkinsonism patches double-stained for calcineurin (C) and neuropeptide Y (D). Asterisks indicate X-linked dystonia-parkinsonism patches. (E and F) Merged images of an X-linked dystonia-parkinsonism patch (E) and the inter-patch area (F) double-stained for calcineurin (green) and neuropeptide Y (red). Neuropeptide Y-positive cells are indicated by open circles. (G) The percentage of the population of neuropeptide Y-positive cells examined (n = 500) in the X-linked dystonia-parkinsonism (XDP) patches and inter-patch area in striatal sections doubly-stained for neuropeptide Y and calcineurin from each X-linked dystonia-parkinsonism patient (n = 4). Values are means ± SEM. N.S. = no significant difference (P > 0.05, Mann-Whitney U-test) between X-linked dystonia-parkinsonism patches and inter-patch area. CN = caudate nucleus; PUT = putamen; NA = nucleus accumbens.
X-linked dystonia-parkinsonism is a transcriptional dysregulation syndrome with impaired expression of the TAF1 gene (Nolte et al., 2003; Makino et al., 2007). TAF1 is the largest subunit of transcription factor IID (TFIID), the binding of TFIID to the core promoter elements is required for assembly of a functional transcription initiation complex (Wassarman and Sauer, 2001). We previously found a significant decrease in the expression of a neuron-specific isoform of the TAF1 gene, named N-TAF1, in the brains of patients with X-linked dystonia-parkinsonism (Makino et al., 2007). N-TAF1 is thought to play an essential role in neuronal survival through transcriptional regulation of multiple neuron-specific genes (Makino et al., 2007; Jambaldorj et al., 2012), whereas the pathomechanism by which the loss of N-TAF1 results in striatal neurodegeneration associated with the occurrence of dystonia remains to be elucidated.

X-linked dystonia-parkinsonism is manifested in adulthood, usually in the third to fourth decade of life (Lee et al., 1976, 2002). It is manifested predominantly as dystonia that spreads and becomes generalized, and then becomes less severe or is replaced by parkinsonism (Lee et al., 2002). We previously carried out post-mortem analyses to show a clinicopathological correlation between dystonia and parkinsonism phenotypes in patients with X-linked dystonia-parkinsonism (Goto et al., 2005). The striosome, but not matrix, compartment shows severe loss of medium spiny neurons during the earlier stages of X-linked dystonia-parkinsonism, when dystonia is the predominant manifestation. Given the hypothesis

![Figure 9](image-url) The loss of neuropeptide Y-positive cells in the neostriatum of patients with X-linked dystonia-parkinsonism. (A–C) Loss of neuropeptide Y-positive cells in the caudate nucleus in patients with X-linked dystonia-parkinsonism. Representative microscopic images of the caudate nucleus stained for neuropeptide Y in normal individuals (A) and patients with X-linked dystonia-parkinsonism (B). (C) Cell density measurements of neuropeptide Y (NPY)-positive cells in the caudate nucleus (CN) in normal individuals (Controls) and patients with X-linked dystonia-parkinsonism (XDP). Values are means ± SEM (n = 20). *P < 0.001 versus control subjects; Mann-Whitney U-test. (D–F) Loss of neuropeptide Y-positive cells in the putamen in patients with X-linked dystonia-parkinsonism. Representative microscopic images of the putamen stained for neuropeptide Y in normal individuals (D) and patients with X-linked dystonia-parkinsonism (E). (F) Cell density measurements of neuropeptide Y-positive cells in the putamen (PUT) in normal individuals (Controls) and patients with X-linked dystonia-parkinsonism (XDP). Values are means ± SEM (n = 20). *P < 0.001 versus control subjects; Mann-Whitney U-test. (G–I) Sparing of neuropeptide Y-positive cells in the nucleus accumbens in patients with X-linked dystonia-parkinsonism. Representative microscopic images of the nucleus accumbens stained for neuropeptide Y in normal control subjects (G) and patients with X-linked dystonia-parkinsonism (H). (I) Cell density measurements of neuropeptide Y-positive cells in the nucleus accumbens (NA) in normal individuals (Controls) and patients with X-linked dystonia-parkinsonism (XDP). Values are means ± SEM (n = 20). N.S. = no significant difference (P > 0.05, Mann-Whitney U-test) between control subjects and X-linked dystonia-parkinsonism. Scale bars = 100 μm.
that an imbalance in striosomal activity relative to the matrix compartment is an aetiological factor in the development of movement disorders that include chorea and dystonias (Graybiel et al., 2000; Sato et al., 2008; Goto et al., 2010; Crittenden and Graybiel, 2011; Fuchs et al., 2012), a predominant loss of striosomal medium spiny neurons could be an important pathology to explain the development of dystonia in X-linked dystonia-parkinsonism. During the later stages of X-linked dystonia-parkinsonism, when dystonia is less severe and is replaced by parkinsonism, the greater involvement of the matrix compartment causes a severe and critical loss of matrix-based projections, leading to the development of the so-called ‘extra-nigral form’ of parkinsonism, as has been suggested in the late stages of Huntington’s disease (Reiner et al., 1988; Albin et al., 1990) and in multiple system atrophy of parkinsonian type (Sato et al., 2007). In this study, we showed a reduced expression of neuropeptide Y in the striatum of patients with X-linked dystonia-parkinsonism with generalized dystonia. This novel finding may be important for understanding the pathomechanism that underlies not only striatal neurodegeneration but also dystonia genesis in X-linked dystonia-parkinsonism, since neuropeptide Y has a potential role in modulating glutamate and dopamine release in the brain (Xapelli et al., 2006; Noe et al., 2008; Woldbye et al., 2010; Decressac and Barker, 2012; van den Pol, 2012). Maladaptive synaptic plasticity at glutamatergic synapses coupled with dopamine receptors in striatal medium spiny neurons has been implicated in the development of levodopa-induced dyskinesia in Parkinson’s disease (Calabresi et al., 2010; Feyder et al., 2011; Murer and Moratalla, 2011), which could share a common molecular abnormality with dystonia (Fuchs et al., 2012). Potential dysregulation of glutamate and dopamine signalling caused by neuropeptide Y loss may also contribute to dystonia genesis in X-linked dystonia-parkinsonism.

Figure 10 The lack of neuropeptide Y labelling and proliferating cell nuclear antigen-positive cells in the subventricular zone of patients with X-linked dystonia-parkinsonism. (A and B) Graded colour-converted images of the striatum stained for neuropeptide Y from a normal individual (A) and a patient with X-linked dystonia-parkinsonism (B). Arrows indicate the subventricular zone. Note a heightened labelling for neuropeptide Y in the subventricular zone in (A) and its absence in (B). (C and D) Photomicrographs of the subventricular zone stained for neuropeptide Y from a normal individual (C) and a patient with X-linked dystonia-parkinsonism (D). (E and F) Photomicrographs of the subventricular zone stained for proliferating cell nuclear antigen from a normal individual (E) and a patient with X-linked dystonia-parkinsonism (F). (G) High power-magnified image of proliferating cell nuclear antigen-positive nuclei in the subependymal zone of a normal individual. (H) Cell count analysis on the numbers of proliferating cell nuclear antigen (PCNA)-positive cells in the subependymal zone in normal individuals (Controls) and patients with X-linked dystonia-parkinsonism (XDP). Values are means ± SEM (n = 4).

*P < 0.001 versus control subjects; Mann-Whitney U-test. Scale bars: A and B = 4 mm; C and D = 1 mm; E and F = 200 μm; G = 10 μm. EL = ependymal layer; SEZ = subependymal zone.
In the adult mammalian brain, the subventricular zone serves as a persistent germinative zone that generates new progenitor cells that can migrate and differentiate within the striatum affected by neurodegeneration (Curtis et al., 2007) and cerebral ischaemia (Arvidsson et al., 2002; Parent et al., 2002; Jin et al., 2006). Experimental evidence has shown that neuropeptide Y plays a role in subventricular zone neurogenesis during development and in adult life (for review see Curtis et al., 2007; Decressac and Barker, 2012; Malva et al., 2012). Neuropeptide Y stimulates proliferation of subventricular zone precursor cells by exerting a mitogenic action through a neuropeptide Y1 receptor-mediated mechanism (Hansel et al., 2001; Agasse et al., 2008; Stanic et al., 2008; Decressac et al., 2009; Thiriet et al., 2011). Interestingly, it was shown that in mice, intraventricular administration of exogenous neuropeptide Y had a striking proliferative effect on subventricular zone neuroblasts and the subventricular zone-derived neurons could migrate toward the striatum where they preferentially differentiate into a major subclass of medium spiny neurons expressing GABA and DARPP-32 (now known as PPP1R1B) (Decressac et al., 2009). In patients with Huntington’s disease, neuropeptide Y-expressing cells are spared, and their number (Dawbarn et al., 1985) and neuropeptide Y immunoreactivity (Beal et al., 1988) are increased in the striatum. As the subventricular zone of patients with Huntington’s disease is enriched in neuropeptide Y-positive cells and progenitor cells labelled for proliferating cell nuclear antigen, an increase in neurogenesis has been suggested in Huntington’s disease (Curtis et al., 2003, 2005, 2007). In contrast, as shown in this study, patients with X-linked dystonia-parkinsonism exhibit a significant loss of neuropeptide Y immunoreactivity in both the neostriatum and subventricular zone, and a marked decrease in the number of proliferating cell nuclear antigen-positive cells in the subependymal zone. Taken together, we suggest that patients with X-linked dystonia-parkinsonism probably lack the ability to generate the subventricular zone progenitor cells depending on the striatal neuropeptide Y activity. If our assumption is correct, neuropeptide Y or neuropeptide Y1 receptor agonists could serve as potential therapeutic tools in attenuating progressive loss of striatal medium spiny neurons in patients with X-linked dystonia-parkinsonism, since neuropeptide Y could promote the self-repair capacity of the adult brain by recruiting the endogenous pool of progenitors (for a review, see Decressac and Barker, 2012).

In conclusion, we have reported on the striatal neuropeptide Y system in the human brain and defects in this system in patients with X-linked dystonia-parkinsonism. Our results may give rise to a new insight in understanding the pathomechanism by which a progressive loss of striatal medium spiny neurons causing dystonia symptoms occurs in X-linked dystonia-parkinsonism. We suggest that X-linked dystonia-parkinsonism could serve as a human disease model to study the role that neuropeptide Y plays in neuroprotection and neurogenesis in the adult human brain.

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References


Feyder M, Bonito-Oliva A, Fisone G. L-DOPA-induced dyskinesias and abnormal signaling in striatal medium spiny neurons: focus on dopamine D1 receptor-mediated transmission. Front Behav Neurosci 2011; 5: 71.


Tepper JM, Tecuapetla F, Koos T, Ibanez-Sandoval O. Heterogeneity and diversity of striatal GABAergic interneurons. Front Neuroanat 2010; 4: 150.


