Genetic study of an American family with DYT3 dystonia (lubag)

Hao Deng, Wei-Dong Le, Joseph Jankovic

Department of Neurology, Baylor College of Medicine, USA
Center for Experimental Medicine, the Third Xiangya Hospital, Central South University, China

Abstract

X-linked dystonia-parkinsonism (XDP, DYT3 [MIM 314250]), endemic with a frequency of 5.24/100,000 in the Philippine island of Panay, is characterized by the clinical onset with dystonia followed by parkinsonism. We found a 35-year-old American male patient, originally from Panay with typical XDP, has a 2-year history of parkinsonism, dystonia, and tremor. Ancestral DYT3 haplotype and disease-specific SVA (short interspersed nuclear element, variable number of tandem repeats, and Alu composite) retrotransposon insertion were identified in the DYT3 proband and two female unaffected family members. No mutation(s) and expression changes in peripheral blood lymphocytes were observed in the TATA-binding protein-associated factor 1 gene (TAF1) or the chemokine CXC motif receptor 3 gene (CXCR3) of the proband or other DYT3 carriers. These findings indicate blood DNA test has a diagnostic utility and implications for genetic counseling in families with DYT3. In contrast, TAF1 and CXCR3 gene expression in peripheral blood lymphocytes is not a suitable surrogate disease marker for DYT3.

Keywords: Dystonia, Parkinsonism, Tremor, Lubag, X-linked

Received 12 August 2008
Accepted 15 October 2008

© 2008 Published by Elsevier Ireland Ltd.
1 mM MgCl2 (Invitrogen) and 0.4 mM of each primer, described previously [14]. PCR amplifications were sequenced bi-directionally using ABI3100 genetic analyzer (Applied Biosystems Inc., Foster City, CA) for haplotype construction.

Total RNA was extracted from peripheral blood lymphocytes with TRIZOL Reagent (Invitrogen, Carlsbad, CA, USA). Reverse transription of isolated RNA was performed using iScript™ cDNA Synthesis Kit (Bio-RAD, Hercules, CA, USA). The TAF1 gene and the chemokine CXC motif receptor 3 gene (CXCR3) were screened and the transcribed cDNA fragments isolated using paired primers (Table 1). The thermocycling profile was as follows: A three-step PCR (94 °C for 45 s; 59 °C for 45 s; 72 °C for 45 s) for 35 cycles. PCR products were sequenced directly. The whole coding regions of two genes (TAF1 and CXCR3) and promoter region of the TAF1 gene were analyzed by sequencing cDNA and gDNA, respectively with the paired primers (Table 2).

Semi quantitative polymerase chain reaction (semiquantitative-PCR) was used to identify the expression of cDNA and the dose of the target genes relative to β-actin was normalized and adjusted to normal controls. The cDNA expression levels were quantified by densitometric analysis of adjacent disease-causing genes, was found to be associated with decreased expression of this gene in the caudate nucleus [12,15]. This may possibly account for the clinical and morphological abnormalities associated with DYT3, including degeneration of the dopaminergic nigrostriatal pathway [16]. The selective loss of striosomal neurons (with sparing the striatal matrix compartment in the striatum), that normally inhibit nigrostriatal dopaminergic neurons via GABAergic innervation may account for relative dopamine excess in the striatum, which in turn is manifested by a hyperkinetic state or dystonia [8]. DYT3 may thus be considered a developmental disorder in which reduced TAF1 interferes with normal expression of genes essential for the development and maintenance of striatal neurons, such as DRD2 [9].

We found that all three individuals in our DYT3 family, including the proband, two unaffected family members and 60 normal controls in the amplification of the fragments of TAF1 cDNA. The two genes, TAF1 and TAF1L, are highly similar in their sequence. Further genomic sequence analysis of exons 1–3 and upstream –1040 bp of the TAF1 gene in the patient also failed to identify any mutation(s). No significant differences between proband and normal controls were found in lymphocytes TAF1 and CXCR3 gene expression by semiquantitative PCR analysis (p = 0.5548 and p = 0.878, respectively).

In the local dialect of Panay Island, Philippines, DYT3 is referred to as ‘lubag,’ meaning ‘twisted’, highlighting dystonia as one of the most recognizable features [4]. In addition to dystonia, DYT3 is characterized by onset with dystonia followed by parkinsonism. The disease usually starts in the fourth decade of life but the age at onset varies between adolescence and early sixties. Although more than 500 men with DYT3 have been studied to date, a few symptomatic female patients with DYT3 have been reported [4]. The average age at onset for female patients with DYT3 seems to be older than that reported in men (52 vs 39 years) and the features are generally mild. Some brains of patients with this dystonia-parkinsonism syndrome have been found to have a mosaic pattern of striatal gliosis [20].

The gene causing this form of inherited dystonia has been mapped to the pericentromeric region of the X-chromosome, Xq13.1 [14]. A multiple transcript system involved in the TAF1 gene was reported to be associated with DYT3 phenotype and TAF1 is designated as the DYT3 gene. It has been suggested that extreme X-inactivation likely underlies the disease in a subset of women carriers [4]. Ten genes including NLGN3 (neuroligin 3), GJB1 (gap junction protein, beta-1), ZNF261 (zinc finger protein 261), NONO (non-POU domain–containing octamer-binding protein), ITGB1BP2 (melusin [integrin beta-1 binding protein 2]), TAF1, ING2 (inhibitor of growth 2), OGT (O-linked N-acetylglucosamine transferase), ACRC (acid repeat–containing gene), and CXCR3 are located in the DYT3 critical region. We conducted mutation analysis of TAF1 and CXCR3 coding region, and excluded escape detection of disease-caused mutation(s) reported in some previous studies [12,14]. Although Nolte et al. reported five DSCs and a 48 bp deletion unique to DYT3, PCR technique used in their cases failed to identify large sequence variants such as transposons. Recently, a SVA retrotransposon insertion in intron 32 of the TAF1 gene, thought to be active in the human genome as well as alter the expression level of adjacent disease-causing genes, was found to be associated with decreased expression of this gene in the caudate nucleus [12,15]. This may possibly account for the clinical and morphological abnormalities associated with DYT3, including degeneration of the dopaminergic nigrostriatal pathway [16]. The selective loss of striosomal neurons (with sparing the striatal matrix compartment in the striatum), that normally inhibit nigrostriatal dopaminergic neurons via GABAergic innervation may account for relative dopamine excess in the striatum, which in turn is manifested by a hyperkinetic state or dystonia [8]. DYT3 may thus be considered a developmental disorder in which reduced TAF1 interferes with normal expression of genes essential for the development and maintenance of striatal neurons, such as DRD2 [9].

We found that all three individuals in our DYT3 family, including the proband, the unaffected mother and her sister, harbored

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5’ → 3’)</th>
<th>Reverse primer (5’ → 3’)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAF1</td>
<td>ATATTCCCCAGTGCACCTCAG</td>
<td>GCCAGCTGGTATGGGCTGTT</td>
<td>110</td>
</tr>
<tr>
<td>CXCR3</td>
<td>AGGCCCTCCCTTCTGCTG</td>
<td>CAGGAGCTTGCTGCTACCA</td>
<td>128</td>
</tr>
<tr>
<td>β-actin</td>
<td>AAGCCATTAGCCCTCACAGAT</td>
<td>AGAAAATTCGCGACGACACC</td>
<td>265</td>
</tr>
</tbody>
</table>
the disease-specific haplotype and SVA retrotransposon insertions. The two female carriers (III:1 and IV:3) were free of any neurologic deficit. SVA retrotransposon insertions are thought to be active in the human genome and to alter the expression level of adjacent genes that cause diseases including Fukuyama-type congenital muscular dystrophy (FCMD [MIM 607440]) and autosomal recessive hypothyroidism (ARH [MIM 605747]) [10]. It is possible that the disease mechanism of DYT3 involves Alu elements in retrotransposon insert that are microRNA targets, regulating the expressions of nearby genes [15].

Because of obvious problems in obtaining brain tissue, progress in genomics of neurological diseases has been slow. Blood genomic and RNA expression profiling, however, are emerging as important techniques used to elucidate disease mechanisms and to identify surrogate disease markers with possible utility in future diagnosis and treatment decisions [3,17]. To evaluate the gene expression changes involved DYT3 in blood, the *TAF1* and *CXCR3* gene expressions were analyzed. No significant differences in gene expression were found comparing with patient and controls (>0.05). This is, to our knowledge, the first report of blood RNA expression profiling in American Caucasian early-onset Parkinson disease families, and PINK1 genes in American Caucasian early-onset Parkinson disease families, the PINK1 induces apoptosis in dopaminergic cells SH-SY5Y, Biochem. Biophys. Res. Commun. 337 (2005) 1113–1118.

In conclusion, in our Phillipino DYT3 family, we identified specific genetic markers DSCs haplotype and SVA retrotransposon insertion, which may be used as a routine molecular genetic diagnosis of DYT3 patients. In contrast blood *TAF1* and gene *CXCR3* expression profiling may not reliable surrogate markers for this disorder.

**Acknowledgments**

The authors thank the participating individuals for their cooperation and Christopher Kenney, MD for referring the proband. Supported by grants from NS 043567 (W.L.) from the National Institute of Neurological Disorders and Stroke, the National Parkinson Foundation to the Baylor College of Medicine Center of Excellence, Diana Helis Henry Medical Research Foundation, Parkinson’s and Movement Disorder Foundation (H.D.), Sheng Hua Scholars Program and Out-standing Youth Foundation of Central South University, China (H.D.), and National Natural Scientific Foundation of China (No.30871351, H.D.).

**References**


