...n a long RT-PCR method is known to be effective for defining the extent of a large transcript.[11] The two ...vealed not only the extent of the transcripts but also alternative exons included in the transcripts. Long RT was ...rformed using TAF_r_7621 for the TATA-binding protein-associated factor 1 gene (*TAF1* [MIM 313650]) and MTS_r for *MTS* as the long RT primer.

Quantitative RT-PCR

cDNA was synthesized from the total RNA by use of random hexamers with a TaqMan Reverse Transcription Reagents kit (Applied Biosystems). All the primers and probes are listed in table 4. We also employed a control probe for 18S rRNA (4319413E) and the glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) (4310884E) and examined a probe for the dopamine receptor D2 gene (*DRD2*) (Hs00156514_m1). We used a final concentration of 250 nM probe, 900 nM primers, and 50 ng cDNA in a 50-μl reaction volume in a 96-well reaction plate on an ABI PRISM 7000 in accordance with the standard procedure. The conditions of real-time PCR consisted of a holding step for 2 min at 50°C and 1 min at 95°C followed by 50 cycles for 15 s at 95°C and 1 min at 60°C. Quantity was calculated every time by use of a standard curve for each well. All quantitative data normalized by adjustment for 18S rRNA were tested by Smirnov's test with a 5% significance level. We used Student's *t* test to test the difference in means of the expression levels between patients with XDP and healthy control caudate nuclei, after checking the acceptance of the quality of variances between the two groups by the *F* test.

Table 4.

Primers and Probes for the TaqMan Expression Assay

In Situ Hybridization

Synthesis of DIG-labeled riboprobes for *TAF1* (probe 3 in fig. 2*a*), as well as for β-actin (*ACTB*) and glial fibrillary acidic protein (*GFAP*) as controls, was performed according to the procedure used for northern analysis. The caudate blocks were fixed with 4% paraformaldehyde. After cryoprotection, serial 20-μm sections were cut in a cryostat. The sections were reacted with alkaline phosphatase–labeled anti-DIG antibody (diluted 1:200 [Roche Diagnostics]) in 1% skim milk in sodium Tris (hydroxymethyl)-aminomethane (NT) buffer. After washing with NT buffer, the positive signals were detected by nitroblue tetrazolium chloride (Roche Diagnostics) and 187 μg/ml 5-bromo-4-chloro-3-indolyl-phosphate (Roche Diagnostics).

Figure 2.

Northern analysis. *a,* Three probes for northern hybridization to *TAF1* (detailed information on these probes is given in table 3). *b,* Total RNA samples from the caudate and lymphoblastoid tissues. The hybridization signal seen at ~7 kb, which represents ...

Immunohistochemical Staining

The six tissues from six different patients with XDP were fixed in 10% neutral formalin, were sliced, and were embedded in paraffin, and 3-μm sections were cut on a microtome and were mounted on Matsunami adhesive silane–coated glass slides. After routine deparaffinization, rehydration, and blocking of endogenous peroxidase activity, all sections were processed for microwave-enhanced antigen retrieval. The sections were blocked with 3% BSA in PBS (pH 7.2) for 1 h and then were incubated overnight at room temperature in 3% BSA-PBS containing goat polyclonal antibody against *TAF1* (diluted 1:5,000 [Santa Cruz]). Rabbit polyclonal antibody against *GFAP* (Dako) was also used as a control. For the visualization of bound antibodies, we used Histofine Simplestain Max-PO (G) (Nichirei) and the liquid