.ated from these BAC libraries. A shotgun library in pUC118 was constructed from each BAC clone. Cycle .equencing was performed using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and was analyzed on an ABI 3700 capillary sequencer. The gap clones were sequenced using a GPS-1 Genome Priming System (New England Biolabs). Raw sequence data were analyzed and assembled by ATGC software (Genetyx).

We digested 4 μg of genomic DNA in 300 μl of a standard reaction mixture containing 125 units of *Hin*dIII for 3 h at 37°C. For methylation detection, an aliquot of the *Hin*dIII-digested DNA was digested under the same conditions with a CpG methylase–sensitive enzyme, *Hpa*II. As a control reaction, an aliquot was also digested with *Msp*I, which is a CpG methylase–insensitive isoschizomer of *Hpa*II. After ethanol precipitation, the digested DNA was loaded onto a 0.8% agarose gel in 0.1× Tris-borate-EDTA buffer for electrophoresis. After denaturation and the subsequent neutralization steps, the DNA was transferred to a positively charged nylon membrane (Roche Diagnostics). The filter was prehybridized for 1 h in DIG Easy Hyb buffer (Roche Diagnostics) and then was hybridized overnight (16–18 h) at 40°C. The PCR probe was amplified using primers XD_probe-F (5′-AGCTTTGCTGCCATTG-3′) and XD_probe-R (5′-AAGACCCTTATTATTCATGAGTG-3′). After washing the filter for 30 min at 68°C in DIG Wash and Block buffer, drops of 1/100-diluted disodium 3-(4-methoxyspiro {1,2-dioxetane-3,2′-(5′-chloro) tricyclo [3.3.1.1^{3,7}]decan}-4-yl) phenyl phosphate (CSPD [Roche Diagnostics]) were added and the filter was exposed to x-ray film for 1–5 h.

RNA Isolation

Total RNA was isolated from caudate, cortex, and accumbens of a frozen brain by use of an RNeasy Lipid Tissue Midi kit (QIAGEN) with a DNaseI treatment step, after homogenization with a Polytron PT1300D (Kinematica). Total RNA from lymphoblastoid cell lines was also isolated by the same procedure. RNA sources from six Japanese brains were used as neurologically healthy controls by the same procedure. Also, two commercial human brain RNA sources were used as controls—cerebral cortex total RNA (catalog number 636561) and caudate nucleus total RNA (63566)—along with human tissue total RNA sources from heart (64100), spleen (64093), lung (64092), liver (64099), and thymus (64107), provided by BD Bioscience Clontech, and stomach (735038), provided by Stratagene. The quality and quantity of total RNAs were assessed using an Agilent 2100 Bioanalyzer (Agilent). The 2:1 ratio of 28S:18S rRNA was employed as a threshold for intact RNA. The quantity was confirmed by the RiboGreen RNA fluorescence assay (Molecular Probes).

Northern Analysis

For synthesis of riboprobes, we performed PCR and cloning into the TA-vector (Promega), using the three primer sets in table 3: TA_f_2333 and TA_r_2690 for probe 1, TA_f_4786 and TA_r_5185 for probe 2, and TA_f_5637 and TA_r_6065 for probe 3. Total RNA samples of 10 μg were loaded into a 1% agarose gel denatured by 2% formaldehyde gel. The gel was run in 1× 3[N-Morpholino]propanesulfonic acid buffer. After electrophoresis, the RNA was transferred to a positively charged nylon membrane. After prehybridization for 1 h in DIG Easy Hyb buffer at 68°C, hybridization was performed overnight (16–18 h) at 68°C. The filters were washed twice for 5 min with 100 ml of 2× saline sodium citrate (SSC) and 0.1% SDS at room temperature and then were washed twice for 15 min with 0.1× SSC and 0.1% SDS at 68°C. We employed the standard conditions and procedure provided by Roche Diagnostics.

Table 3.

PCR Primer Sets for Fragment Amplification from the *TAF1* cDNAs[Note]

Long RT-PCR Analysis

Long RT-PCR analysis was performed in two steps: (1) first-strand synthesis from RNAs of the control and XDP caudates by long reverse transcription (RT) and (2) fragment PCR by use of the long RT products (i.e., cDNA) as a