Information about the gene expression profiling data

in *BrainTx* (formerly CDT-DB)

1. Fluorescent differential display (FDD) analysis

We analyzed mRNA expression in ICR mouse cerebella during postnatal developmental (stages E18, P0, P3, P7, P12, P15, P21, and P56) by FDD. DNase-treated total RNAs isolated from each stage were subjected to RT-PCR in duplicate, using combinations of arbitrary primers (10-mer) and FITC-labeled anchor poly-d(T) primers (GT(15)X; X = A, C, or G), followed by gel electrophoresis under non-denaturing conditions. FITC-labeled bands with differential developmental patterns were visualized using a fluorescent image scanner (Molecular Imager FX, Bio-Rad Laboratories) and were excised from gels, followed by cloning into TA-cloning vectors such as pCR4-TOPO (Invitrogen). One-run DNA sequencing analysis of about 12,000 FDD clones was carried out at the facilities of the RIKEN Genomic Sciences Center (in collaboration with the Genome Sequencing Team). As a result of BLAST searches, about 2,000 non-redundant FDD clones were identified. It should be noted that some FDD clones contain sequences outside the exons of known or predicted genes, for example, introns, the 5'-flanking region, and the 3'-flanking region, as described above, although RNA samples were treated with RNase-free DNase to remove contaminated chromosomal DNAs.

2. cDNA microarray (CDT array) analysis

We generated a custom-made cDNA microarray (named "**CDT array**") spotted with about 2,400 genes that were either represented by the FDD clones or known to be involved in cerebellar development. We applied CDT array analysis to parallel monitoring of the expression of these CD genes during cerebellar development (stages E18, P0, P3, P7, P12, P15, P21, and P56) in mice (ICR). Hybridization was carried out using probes labeled with Cy3 and Cy5 (CyScribe, Amersham). Digital images of hybridization were acquired using a laser scanner (GenePix4000A, Axon Instruments) and analyzed with bioinformatics software (Acuity 3.1, Axon Instruments, or GeneSpring GX v10, Agilent). In the CDT-DB, the CDT array data are available in graphical formats showing the expression profiles of single genes or groups of genes within gene categories. We also analyzed gene expression profiles in the cerebella of five spontaneous mutant mice, *Lurcher, Purkinje cell degeneration (pcd), reeler, staggerer, and weaver*, data on which will be updated in the future.

3. Affymetrix GeneChip analysis

Developmental time series GeneChip analysis- We first analyzed gene expression profiles during cerebellar development of ICR mice (E18, P7, P14, P21, and P56) by utilizing the GeneChip system (Affymetrix Mu11K, 12,654 probes, including known mouse genes and ESTs). We found that 10,321 probes (81.6%) were expressed in one of the developmental stages, and that 8.7% of these (897/10,321) showed apparent differential expression during development, in which the difference between the highest and lowest expression signals was more than two-fold (see 4. Publication-3, Kagami and Furuichi, 2001). In the CDT-DB, we have compiled GeneChip data, including data on five additional probe genes (for a total of 902), in graphical formats of single genes or groups of genes within gene categories.

Some of these data are also available in the NCBI Gene Expression Omnibus (GEO) (Platform GPL8, Series GSE2, and Sample GSM50, GSM51, GSM52, GSM53, and GSM54).

- GPL8
- GSE2
- GSM50
- GSM51
- GSM52
- GSM53
- GSM54

To increase coverage for the detection of the CDT, we recently utilized the Affymetrix Mouse Genome 430 2.0 Array, including probes for about 39,000 transcripts, for the analysis of C57BL/6J mice. As a result, approximately 7,100 probes were identified as differentially expressed during cerebellar development (see 4. Publication-1, Sato et al., 2008).

Tissue specific GeneChip analysis- To estimate the brain specificity of gene expression, we utilized the Affymetrix Mouse Genome 430A 2.0 Array, including probes for approximately 15,000 transcripts, and analyzed RNAs from eight different tissues (brain, thymus, lung, heart, liver, spleen, kidney, and testis) of mice (C57BL/6J) at P7 or P21.

4. RT-PCR analysis

Developmental time series RT-PCR- We analyzed the developmental time series expression patterns of CD genes by a conventional semi-quantitative RT-PCR method using ExTaq polymerase (Takara), cerebellar cDNAs of eight developmental stages (E18, P0, P3, P7, P12, P15, P21, and P56), and CD gene-specific primer sets (18-25 mer) at 20-35 cycles in a GeneAmp9700 thermal cycler (PerkinElmer). After agarose gel electrophoresis, PCR products were stained with EtBr, and digital images of banding patterns were acquired using a fluorescent image scanner (Molecular Imager FX, Bio-Rad Laboratories). RT-PCR profiles were tested by multiple experiments (some were tested more than ten times), using different RNA or cDNA sources, or using different primer sets or reaction conditions (temperature, Mg^{2+} concentration, etc.), depending on the consistency of the banding patterns.

Tissue specific RT-PCR- The brain specificity (tissue distribution) of CD gene expression was also analyzed by RT-PCR using cDNAs prepared from RNAs obtained from eight mouse tissues (brain, thymus, lung, heart, liver, spleen, kidney, and testis) at P7 or P21, as described above.

5. *In situ* hybridization (ISH) analysis

The spatial cellular expression patterns of the CD genes were analyzed by *in situ* hybridization (ISH) histochemistry of P7 and P21 mouse brains (ICR or C57BL/6J). Sagittal paraffin sections (6 µm) were prepared using an automated paraffin sample preparation instrument (Tissue-Tek tissue processor and tissue embedding console IV, Sakura) and a rotary microtome with a sampling apparatus (MICROM HM 335E). After proteinase K digestion, hybridization was carried out in a solution containing 4x SSC, 50% formamide, 0.5 mg/ml yeast tRNA, 1x Denhardt's solution, 5 mM EDTA, and digoxigenin (DIG)-UTP (Roche)-labeled riboprobes at 60-65°C. Probes hybridized with mRNAs were detected by staining with anti-DIG antibody (Roche) and NBT/BCIP (Roche). At least two sections prepared from different brain positions were analyzed for each ISH reaction. ISH profiles for each CD gene were generally examined several times, if necessary by changing experimental conditions (temperature, probe or antibody concentration, hybridization or staining time).

In addition to the manual methods described above, we utilized an automated ISH experiment system, Freedom EVO GenePaint (TECAN), developed by Dr. Gregor Eichele's Laboratory at the Max Planck Institute and Bayer College of Medicine, Germany. Some of our ISH data were obtained using this TECAN ISH apparatus and a protocol modified for paraffin sections and regular DIG labeling method without tyramide signal amplification.

Digital images of hybridized sections were acquired using a Nikon E800 microscope equipped with a Spot Insight QE-cooled CCD camera, an Olympus BX51 microscope equipped with a ProgRes C14 cooled CCD camera, and a stereoscopic microscope (Nikon SMZ-U) equipped with a CCD camera (Spot Insight). To obtain high-resolution images of whole mouse brains, we utilized a digital slide scanner NanoZoomer Digital Pathology (NDP; Hamamatsu Photonics K.K.). These high resolution brain images are used to display magnified images in two viewer functions; "Detailed viewer" and "Simple zoom" (see "Help"). If necessary, digital images were adjusted as little as possible using Adobe Photoshop CS2 software to normalize some of the differences (for example, in color, contrast, or scanning noise and moire) that often occur during digitization.