

The Mouse Brain Transcriptome by SAGE: Differences in Gene Expression between P30 Brains of the Partial Trisomy 16 Mouse Model of Down Syndrome (Ts65Dn) and Normals

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Trisomy 21, or Down syndrome (DS), is the most common genetic cause of mental retardation. Changes in the neuropathology, neurochemistry, neurophysiology, and neuropharmacology of DS patients' brains indicate that there is probably abnormal development and maintenance of central nervous system structure and function. The segmental trisomy mouse (Ts65Dn) is a model of DS that shows analogous neurobehavioral defects. We have studied the global gene expression profiles of normal and Ts65Dn male and normal female mice brains (P30) using the serial analysis of gene expression (SAGE) technique. From the combined sample we collected a total of 152,791 RNA tags and observed 45,856 unique tags in the mouse brain transcriptome. There are 14 ribosomal protein genes (nine underexpressed) among the 330 statistically significant differences between normal male and Ts65Dn male brains, which possibly implies abnormal ribosomal biogenesis in the development and maintenance of DS phenotypes. This study contributes to the establishment of a mouse brain transcriptome and provides the first overall analysis of the differences in gene expression in aneuploid versus normal mammalian brain cells.

Trisomy 21 (T21), or Down syndrome (DS), is one of a few autosomal aneuploidies in which many cases escape from fetal or early postnatal lethality. DS phenotypes show variable penetrance, affecting many different organs including brain (mental retardation, early-onset Alzheimer's disease), muscle (hypotonia), heart (congenital heart defects), skeleton (short stature; short fifth finger; short, broad hands; brachycephaly), and blood (increased occurrence of leukemia). However, of these abnormalities, only mental retardation is invariably present in all DS patients (Epstein 1995).

The chromosomal abnormality underlying the DS phenotype, the presence of three copies of chromosome 21 (HC21), was first described in 1959 (LeJeune et al. 1959). Several studies have been undertaken to analyze possible alterations of mRNA or protein levels caused by the presence of an additional set of HC21

genes (Weil and Epstein 1979; Van Keuren et al. 1982; Whatley et al. 1984); however, the precise molecular mechanism that is responsible for the development and maintenance of the complex DS phenotypes remains unclear. The generally accepted working hypothesis is that HC21 contains a certain number of genes that, if present in three copies, will contribute to the DS phenotypes. The accomplishment of the entire sequence of HC21 has been recently reported (Hattori et al. 2000). However, the various DS phenotypes may stem directly from the overexpression of HC21 gene products or indirectly from the interaction of these HC21 gene products with the whole genome, transcriptome, and proteome.

Three viable mouse models that bear three copies of part of mouse chromosome 16 (MMU16), which is homologous to HC21, have been produced: Ts65Dn mice contain three copies of MMU16 from *App* to *Mx1* (Reeves et al. 1995); Ts1Cje mice have three copies of MMU16 from *Sod1* to *Mx1* (Sago et al. 1998a); and Ms1Ts65 mice have three copies of MMU16 from *App* to *Sod1* (Sago et al. 1998b). All of these mice show learning and behavioral abnormalities. The phenotype is more pronounced in the Ts65Dn mice, which have the largest region partial trisomy 16 (Hernandez and Fisher 1999; Baxter et al. 2000).

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Because mental retardation of different severity is present in all DS patients, we have used the Ts65Dn mice to analyze the global changes of gene expression in the brain of these mice as compared with normal littermates. Gene expression was analyzed in the brains of 30-day-old (P30) Ts65Dn males, normal male and normal female littermates using serial analysis of gene expression (SAGE) methodology (Velculescu et al. 1995). SAGE is a technique that allows the quantitative analysis of a large number of transcripts in a given mRNA population by counting the presence of each transcript. SAGE identifies a short nucleotide sequence of 14–16 bp from a defined position within the transcript (e.g., the 3′-most *Nla*III restriction enzyme site). We describe here a mouse brain transcriptome from >150,000 s—50,000 each from Ts65Dn males, normal males, and normal females. We also report changes in gene expression in Ts65Dn mice compared with normal nontrisomic controls.

Our analysis of the mouse brain transcriptome revealed 45,856 unique tags. Comparison of normal and Ts65Dn male brain SAGE libraries showed that there are 330 tags with statistically significant differences between these two samples. The most frequently altered classes of genes are ribosomal proteins. These data provide the first global differential gene expression study in aneuploid versus normal mammalian brains. This may lead to the discovery of genes that are “directly” involved in several phenotypes of DS and eventually to the identification of potential targets for therapeutic interventions. The mouse brain transcriptome provides a database for comparisons to analyze changes in the spatial and temporal expression patterns of individual genes and global gene expression in other mouse models of human diseases affecting the central nervous system. It may also be helpful in gene expression studies in events, such as changes in behavioral state, onset of disease, and response to drugs.

RESULTS

Mouse Brain Transcriptome

We have sequenced 54,599 tags from control male, 52,167 tags from control female, and 51,450 tags from Ts65Dn male mice SAGE libraries. After elimination of tags that are derived from linkers and duplicate ditags, we have obtained 51,561, 50,726, and 50,504 tags from these SAGE libraries, respectively. Thus the total number of tag sequences was 152,791, corresponding to 45,856 collected tags. SAGE analysis of yeast (Velculescu et al. 1997), for which the entire genome sequence is known, showed a sequencing error rate of ~0.7% per base pair, which translates to a tag error rate of 6.8%; thus there are likely to be 42,738 unique tags represented by the 45,856 collected tags. As shown in Figure 1, we have not reached saturation in unique tag

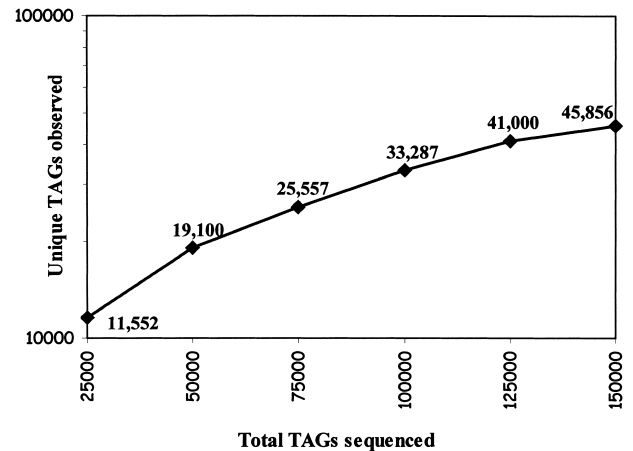


Figure 1 Cumulative curve of unique tag detection through sequencing of 152,791 tags from normal male and Ts65Dn male and normal female mice brain serial analysis of gene expression (SAGE) libraries. A total of 22,115, 21,357, and 19,103 unique tags were detected after sequencing 51,561, 50,504, and 50,726 tags from normal male, Ts65Dn male, and normal female mice brain SAGE libraries, respectively.

discovery. Indeed, in human colorectal cancer cell lines, unique tag discovery approached zero at ~650,000 tags collected (Velculescu et al. 1999).

Expression levels of mouse genes ranging from two to 5252 transcript copies per cell were observed. Thirty-three transcripts expressed at >500 copies per cell comprised 11% of the cellular mRNA mass and the 748 most highly expressed genes accounted for 37% of the cellular mRNA content (Table 1). Most unique transcripts were expressed at levels equal to or fewer than five copies per cell, with 28% of the mRNA mass comprising 81% of the unique transcripts expressed. The tags matched ~4000 known genes (there are ~6000 mouse mRNA sequences in GenBank) and ~10,000 expressed sequence tag (EST) clusters with unknown functions (out of ~70,000 mouse UniGene clusters), whereas the remaining transcript tags (76%), mainly among the genes with low expression levels, had no matches in public databases (Table 1).

A human SAGE transcriptome derived from 3.5 million transcripts from 19 normal and diseased tissue types was recently published (Velculescu et al. 1999). Comparison of the data between the human and mouse transcriptome shows a similar percentage of cellular transcripts in the various classes of abundance of unique tags (Table 1). Comparison of the 15 most abundant mouse brain transcripts with human brain SAGE data (Velculescu et al. 1999) generally revealed similar levels of expression and identities (Table 2).

Detection of Tags that Are Over- or Underrepresented in Ts65Dn Male SAGE Library

To detect tags that are specifically over- or underrepresented in Ts65Dn male SAGE library, we have com-

Table 1. Comparison of Results of Mouse Brain and Human Transcriptome

Copies/cell		Mouse brain transcriptome		Human transcriptome (Velculescu et al. 1999)	
		N (% of total)	% of cellular transcripts	N (% of total)	% of cellular transcripts
>500	Unique TAGs	33 (0.07%)	11%	55 (0.04%)	18%
	GenBank matches	26 (78%)		55 (100%)	
50–500	Unique TAGs	709 (1.55%)	26%	578 (0.43%)	27%
	GenBank matches	587 (82%)		576 (99%)	
5–50	Unique TAGs	7,821 (17%)	34%	6,160 (4.6%)	30%
	GenBank matches	3,548 (48%)		5,913 (96%)	
≤5	Unique TAGs	37,293 (81%)	28%	127,342 (94.9%)	25%
	GenBank matches	6,927 (18%)		66,091 (52%)	
Total collected TAGs		45,856	100%	134,135	100%
GenBank matches		11,088 (24%)		72,635 (54%)	

Tags were distributed according to their copy number per cell into four categories: >500 copies, 50–500 copies, 5–50 copies, and ≤5 copies per cell (on estimate of 300,000 total transcripts per “average cell” was used). The number of unique transcripts/tags and the corresponding GenBank matches are shown for each category. The percentage of total cellular transcripts contributed by each category is also shown.

pared the individual number of tags in all three libraries (normal males, normal females, and Ts65Dn males) using SAGE analysis software. This analysis revealed 330 statistically significant differences ($P < 0.05$) between normal males and Ts65Dn males, 406 differences between normal males and normal females, and 451 differences between normal females and Ts65Dn males (Figure 2). There were 15 tags with statistically significant differences in common to all comparisons,

which were excluded from further analysis. We have focused on the 330 tags that were detected after comparison of normal and Ts65Dn male libraries (Figure 3). These differences may be a result of the third copy of part of chromosome 16 that is present in the Ts65Dn mice and not the result of the presence or absence of either the Y chromosome or the second copy of the X chromosome (normal male vs. female and normal female vs. Ts65Dn male differences).

Table 2. Comparison of the 15 Most Expressed Tags in the Mouse Brain Transcriptome

TAG sequence	Mouse UniGene no.	pA signal	Human UniGene no.	Mouse gene description	No. of mice TAGs	No. of human TAGs
G T G G C T C A C A	4184	Yes		Multiple matches	2626	
G C T T C G T C C A	2992	Yes	69547	Myelin basic protein	1013	261
A G C A G T C C C C	3776	No		TAG matches mitochondrial sequence	870	
T C A G G C T G C C	1776	Yes	62954	Ferritin heavy chain	573	440
G C A C A A C T T G	18041	No		Multiple matches	554	
G T G A C C T G G C	1008	Yes	8272	Prostaglandin D2 synthase (21 kD, brain)	496	282
G C C T C C A A G G	5289	Yes	195188	Glyceraldehyde-3-phosphate dehydrogenase	490	302
A A A A A A A A A A	15295	Yes		Multiple matches	481	
G A G C G T T T T G	5246	Yes	182937	Peptidyl-prolyl <i>cis</i> -trans isomerase A	475	142
C A A A C C T C C A	28177	No		TAG matches mitochondrial sequence	400	
C A T C G C C A G T	21417	Yes	169401	Apolipoprotein E	397	191
A G C A A T T C A A	30071	No		TAG matches mitochondrial sequence	390	
A A T T C G C G G A				No match	375	
C C T T G C T C A A	43036	Yes	135084	Cystatin C	347	274
T G A C C C C G G G	43766	Yes		Ubiquitin/60s ribosomal fusion protein	333	

The corresponding mRNA to each tag has been determined when possible. The human homolog for most of these was determined using UniGene searches. For each mouse tag sequence the numbers of detected copies in ~150,000 tags from mouse brain SAGE libraries (No. of mice tags) are shown. The presence of polyadenylation signal in the corresponding mouse UniGene entry (Mouse UniGene no.) was noted (pA signal). The corresponding human transcript was obtained from the human UniGene library (Human UniGene no.) and the number of tags representing this transcript in 150,000 tags from human brain SAGE library was determined (No. of human tags). Mouse transcripts with known poly(A) signal for which the homolog was detected in the human brain transcriptome are shaded in gray.

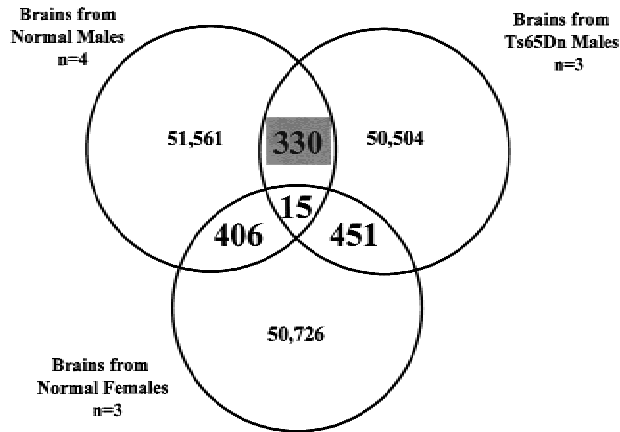


Figure 2 Comparison of ~50,000 tags isolated from serial analysis of gene expression (SAGE) libraries prepared from four normal male, three Ts65Dn male, and three normal female P30 mice brains. We have detected 330 tags with statistically significant differences ($P \leq 0.05$) between normal and Ts65Dn male SAGE libraries, 406 differences between normal male and female SAGE libraries, and 451 differences between Ts65Dn male and normal female SAGE libraries. The complete list of tags that are overrepresented in each sample can be found in the web address <http://medgen.unige.ch/research/projects/SAGE/>.

330 Tag Differences between Ts65Dn and Normal Mouse Brains

Approximately one-half of the differentially expressed tags (161) are underrepresented and one-half are overrepresented (169) in Ts65Dn male compared to normal male SAGE libraries. Of the 330 differences, 112 tags matched known genes, 133 matched ESTs, and 85 were unknown (Table 3; more recent update available at

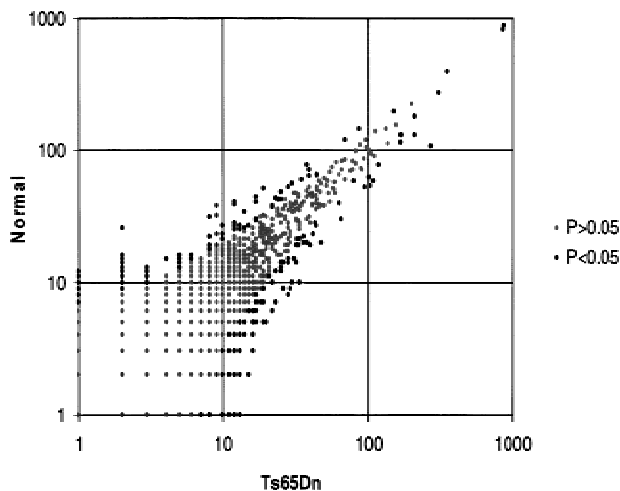


Figure 3 Comparison of the observed frequencies of 36,012 unique tags detected after sequencing of ~51,000 tags from each normal and Ts65Dn mouse serial analysis of gene expression (SAGE) libraries. The vast majority of the tags were expressed at the same or similar level in the two samples (gray circles); however, there were 330 tags with statistically significant differences ($P < 0.05$) between normal and Ts65Dn mouse SAGE libraries (black circles).

<http://medgen.unige.ch/research/projects.html>). We have attempted to organize the 112 differentially expressed tags that match known genes into categories according to their biological function. However, no category including cell cycle, ion channels, apoptosis, DNA synthesis, transcription factors, growth factors, receptors, neurotransmitters, and cytoskeleton was overrepresented. It was shown that apoptosis is responsible for accelerated neuronal death in cultured Ts16 hippocampal neurons (Bambrick and Krueger 1999). Using all apoptosis-related entries by searching functional annotations in SwissProt (<http://www.expasy.ch/srs5/>) and the Clontech mouse Atlas array database (<http://www.clontech.com/archive/JAN98UPD/Atlaslist.html>), we have detected only one differentially expressed gene, the clusterin (GenBank accession no. L08235), which was present by 77 tags in normal male and 38 tags in Ts65Dn male SAGE libraries. The group of ribosomal proteins merits further attention. Among the 245 tags that are homologous with genes and ESTs that are differentially expressed ($P < 0.05$) between normal male and Ts65Dn male brains, there are 14 ribosomal protein genes that are differentially expressed in the two groups (nine underexpressed and five overexpressed; Table 4).

Northern Blot Confirmation of Results

We have attempted to confirm the differential expression of a small number of genes from our SAGE study by Northern blot analyses. Confirmations were performed for the following: (1) transcripts that were overrepresented in the Ts65Dn tag library, such as the *Rbm3* putative RNA-binding protein (seven tags in trisomy and none in normal SAGE library) and the *p19*, 19-kD brain-specific protein (T23/N7); (2) those transcripts that were underrepresented in the Ts65Dn tag library, such as clusterin, an apoptosis-related protein (38 tags in trisomy and 77 tags in normal SAGE library), the *L30* ribosomal protein (14 tags in trisomy and 26 tags in normal SAGE library), and the *L5* ribosomal protein (nine tags in trisomy and 32 tags in normal SAGE library); and (3) for those transcripts that were represented by similar number of tags, such as the cytoskeletal *actin* (57 tags in trisomy and 52 tags in normal SAGE library; Fig. 4). We have confirmed overexpression of *Rbm3* and *p19* and underexpression of *L30* and *L5* ribosomal protein genes in the Ts65Dn sample and shown similar levels of actin mRNA in the normal and Ts65Dn samples. However, we have not been able to confirm underexpression of clusterin in the Ts65Dn samples.

Expression of Genes from the Triplicated Region of Chromosome 16 in Ts65Dn Mice

The Ts65Dn mice have three copies of the region of chromosome MMU16 from markers *App* to *Mx1*

Table 3. Portion of the List of the 330 Tags with Statistically Significant Differences Between Normal and Ts 65 Dn Male Drain SAGE Libraries

TAG sequence	Ts65Dn	Normal	P	GenBank match
TCCCCGACA	2	26	0.0000	No match
AAATTATTGG	110	59	0.0000	Proteolipid protein (myeline)
GCCCCCTCT	104	54	0.0000	No match
TCCCCGACAT	9	38	0.0000	No match
TTGGTGAAGG	19	52	0.0001	Prothymosin β -4
GACTGAATCT	12	39	0.0001	Multiple matches
CGCCCCGCCG	65	30	0.0002	Map1b
ATTTTCGAGG	34	10	0.0002	ATPase, Na ⁺ K ⁺ transporting β 1 polypeptide, Atp1b1
AATTCGCGGA	86	144	0.0002	No match
TCCCCGTACG	8	31	0.0002	No match
GAAAGTTGGC	95	53	0.0002	Apolipoprotein D
TCTCCAGGCG	38	77	0.0003	Clusterin
CTGCTATCCG	9	32	0.0003	Ribosomal protein L5, 3' end
AACGGGAGGT	48	20	0.0003	Scgn10 like-protein
CAGCTCTGCC	70	119	0.0004	ESTs, weakly similar to NEUROGRANIN (<i>Rattus norvegicus</i>)
GACAAACGCCA	17	44	0.0004	ESTs, highly similar to ATP SYNTHASE γ CHAIN, MITOCHONDRIAL (<i>R. norvegicus</i>)
GTGACCTGGC	169	115	0.0004	Prostaglandin D2 synthase (21 kD, brain)
AGGACAAATA	102	62	0.0007	Biotinidase
TCCCCGTAAT	2	16	0.0007	No match
CGCGCTCTCT	29	9	0.0008	No match
CTGCTGGTGG	30	10	0.0009	GTP-binding protein associated protein 1
GCCATCCCCG	13	1	0.0009	No match
CAGGTGAGCC	15	2	0.0010	No match
CGCAGTCCCC	0	10	0.0010	No match
TGGGCGTGGA	0	10	0.0010	No match
GTTGCTGAGA	17	41	0.0014	24.6-kD protein (M93980)
GACGGCTAAA	2	15	0.0015	No match
TCCCCCGTAC	2	15	0.0015	No match
TTTTGAGTGA	12	1	0.0016	No match
GCGCCAGCTC	118	78	0.0016	Ubiquitin C-terminal hydrolase L1
AAACGAAAGT	22	6	0.0017	ESTs, highly similar to S-ADENOSYLMETHIONINE SYNTHETASE γ form (<i>R. norvegicus</i>)
GCGGGCACCA	20	5	0.0017	ESTs
ACAGGAAGGA	1	12	0.0018	No match
ACTTCGTCCA	0	9	0.0019	No match
GAGTCACTCT	0	9	0.0019	ESTs
GCGGGTCGCC	16	3	0.0020	No match
GGGACCTAGG	23	7	0.0023	Neuron-specific gene family member 2
GTCTGGGGGG	2	14	0.0024	Monoglyceride lipase
GCTATGAGAA	26	9	0.0024	Amyloid β (A4) precursor-like protein 1
CCCAGATATT	19	5	0.0028	Tubulin, β -4
GTGGCAGGCG	11	1	0.0029	No match
GTGTGCCCCC	11	1	0.0029	ESTs, highly similar to HYPOTHETICAL 67.8-kD PROTEIN in GND1-ERG9 INTERGENIC REGIONS (<i>Saccharomyces cerevisiae</i>)
GCTTTTGGGA	13	2	0.0034	No match
TGTAGTGTA	8	0	0.0035	Ribosomal protein S8
CCGCCTGTGG	14	34	0.0036	Synapsin I
TCCCCGTGCA	2	13	0.0039	No match
TCTGGTCCGG	1	11	0.0041	Flotillin 2
GCAGAGTGCG	32	14	0.0044	Multiple matches
CCGCCGTGGG	0	8	0.0044	Microtubule-associated protein 7
GTTGTGATTT	0	8	0.0044	Ewing sarcoma homolog
TGAGCACTCC	0	8	0.0044	Follistatin-like
CCTTACCGCT	30	56	0.0046	ESTs
TCCGTGGTTG	28	53	0.0049	ESTs, moderately similar to BIOTIN CARBOXYLASE (<i>Anabaena pcc7120</i>)
TTCTGGCCTG	10	27	0.0050	ESTs, highly similar to EST00098 protein (<i>Homo sapiens</i>)
GAGAGAAGAG	10	1	0.0051	Microtubule-associated protein τ
GGGCAGGGAG	10	1	0.0051	ESTs
GTTTGCTGTG	16	4	0.0053	Cathepsin B
GCGGGTTTCG	12	2	0.0062	No match
TGGTGACCGT	12	2	0.0062	ESTs, weakly similar to similar to kinensin-like protein (<i>Caenorhabditis elegans</i>)

Table 3. (Continued)

TAG sequence	Ts65Dn	Normal	P	GenBank match
ATGAATTTTG	1	10	0.0065	ESTs, highly similar to CELL DIVISION CONTROL PROTEIN 4 (<i>S. cerevisiae</i>)
CCATTGATCA	1	10	0.0065	ESTs, highly similar to bithoraxoid-like protein (<i>R. norvegicus</i>)
CGGATAACCA	1	10	0.0065	Proliferation-associated protein 1
GCTTGTGACG	1	10	0.0065	Transaldolase 1
TTACAGCAGA	1	10	0.0065	ESTs, weakly similar to ORF YGL231c (<i>S. cerevisiae</i>)
TGCAGTCCCC	2	12	0.0065	No match
CCCCAGCCAG	25	48	0.0065	Ribosomal protein S3
ATTGCTTAGA	7	0	0.0070	RNA-binding motif protein 3
CAGTAACTTC	7	0	0.0070	Aquaporin 4
CCCCGCCGCG	7	0	0.0070	Zinc finger protein, multitype 1
CCCCTCCCCA	7	0	0.0070	No match
GGCCTTTGTG	7	0	0.0070	ESTs, weakly similar to coded for by <i>C. elegans</i> cDNA CEESW58F
GGATACGTTT	17	5	0.0075	ESTs
GGCGGGGCCC	17	5	0.0075	ESTs, weakly similar to 230K bullous pemphigoid antigen BPM1 (<i>Mus musculus</i>)
AATCACTGGC	0	7	0.0082	ESTs, moderately similar to protein DS 1, 24K (<i>H. sapiens</i>)
CGACCCCGGG	0	7	0.0082	No match
CTAACCTCCA	0	7	0.0082	No match
GTGTTGTCGG	0	7	0.0082	ESTs, weakly similar to S1-1 protein (<i>R. norvegicus</i>)
TAAAGCAGGAC	0	7	0.0082	No match
CGCTGGTTCC	35	60	0.0087	Replication factor C, 140 kd
AACTGCACAC	13	3	0.0096	ESTs, highly similar to SUCCINATE DEHYDROGENASE (<i>H. sapiens</i>)
GGTCCTGCT	8	22	0.0097	ESTs
CGCGGCAAGG	38	20	0.0104	Dynactin 1
CAGGACTCCG	63	40	0.0106	Stearoyl-Coenzyme A desaturase 2
CTCTTCCCCC	42	23	0.0110	Phosphatidylethanolamine-binding mRNA, complete cds
TCCCCGTACT	4	15	0.0111	No match
ACCAAGATCT	3	13	0.0112	Ribosomal protein S19
ATGGGTATC	3	13	0.0112	ESTs, highly similar to TRANSLOCATION PROTEIN SEC62 (<i>S. cerevisiae</i>)
TCTGCCCTCC	3	13	0.0112	No match
GATTCGCTGA	39	64	0.0113	ESTs, highly similar to 60S RIBOSOMAL PROTEIN L37 (<i>H. sapiens</i> ; <i>R. norvegicus</i>)
GCCAAAAAAA	1	9	0.0113	ESTs, highly similar to 2-OXOGLUTARATE DEHYDROGENASE E1 COMPONENT PRECURSOR (<i>H. sapiens</i>)
GGCTTTGGAG	1	9	0.0113	ESTs, moderately similar to HSPC005 (<i>H. sapiens</i>)
ACTCGGAGCC	23	43	0.0114	Calmodulin
CAAACCTGAAG	2	11	0.0114	ESTs, highly similar to spliceosomal protein SAP 155 (<i>H. sapiens</i>)
CACTACACGG	2	11	0.0114	FK506-binding protein 2 (13 kD)
GGGGCTCAGC	31	15	0.0115	Glucose phosphate isomerase 1 complex
GTAAGTACAT	9	23	0.0116	No match
GCAGCATCTG	16	5	0.0118	ESTs
CTGGCAGAAG	11	2	0.0125	ESTs, highly similar to NIFU PROTEIN [Anabaena pcc7120]
TCTGGAGACA	11	2	0.015	No match
AGAGCTGTGG	14	4	0.0132	NADH dehydrogenase flavoprotein 1
GTGTGTGGTG	19	7	0.0134	ESTs
TGAGAACTGC	19	7	0.0134	Tetratricopeptide repeat domain
TCAGGCTGCC	153	199	0.0135	Ferritin heavy chain
GTGTGCCGCT	17	6	0.0144	No match
AAGCTCAGTT	5	16	0.0146	ESTs
CCACAGAGCT	5	16	0.0146	Ena-vasodilator stimulated phosphoprotein
AAAGTTCGGA	6	0	0.0147	sid23p
ATGCAACTAC	13	27	0.0211	ESTs, highly similar to CYTOCHROME C OXIDASE POLYPEPTIDE VIB (<i>Bos taurus</i>)
TGGGGTTTTCC	44	27	0.0214	Multiple matches
GGGAGCGGGC	22	10	0.0217	ESTs, Highly similar to HYPOTHETICAL 109.5 kD PROTEIN IN PPA1-DAP2 INTERGENIC REGION (<i>S. cerevisiae</i>)
TAAACAGTTGT	13	4	0.0219	Calnexin
AGAGAGGCCA	1	8	0.0226	ESTs, weakly similar to PROFLIN I (<i>M. musculus</i>)
AGGCATTGTC	1	8	0.0226	ESTs
CATCAGCCTC	1	8	0.0226	Multiple matches
CCCAGGCTTG	1	8	0.0226	ESTs
GACTCTTTCT	1	8	0.0226	ESTs
GGCCTCTAAA	1	8	0.0226	ESTs
TGCTGGTGCG	1	8	0.0226	ESTs

Table 3. (Continued)

TAG sequence	Ts65Dn	Normal	P	GenBank match
GCGAGTCCCC	5	15	0.0228	ESTS
TTGGCTCACA	5	15	0.0228	ESTS
GAATTTTAC	16	6	0.0253	No match
GGGCAAGCCA	17	7	0.0268	Estrogen related receptor, α
TGGCTTTTCT	28	15	0.0277	Multiple matches
ATCCTAGGTC	11	3	0.0277	No match
CAGACGGAAG	11	3	0.0277	ESTS
GGGAAAACCT	11	3	0.0277	No match
TCGGCCGCC	11	3	0.0277	<i>M. musculus</i> DNase1 precursor mRNA, complete cds
GGGATGGCCA	8	19	0.0287	ESTS
AATATGTGTG	6	16	0.0298	Cytochrome c oxidase, subunit VIc
AACGCTGCCA	25	42	0.0299	Macrophage migration inhibitory factor
AACGGCTAAC	5	0	0.0302	No match
AACTATTA	5	0	0.0302	ESTS
AAGAGAACCC	5	0	0.0302	No match
AAGCACTGTG	5	0	0.0302	ESTS
AAGTTGCATT	5	0	0.0302	NUCLEOSOME ASSEMBLY PROTEIN 1-LIKE 1
ACCTTGCCCT	5	0	0.0302	ESTS, highly similar to TRANSLOCON-ASSOCIATED PROTEIN, β SUBUNIT PRECURSOR (<i>H. sapiens</i>)
ACTCCCCAGA	5	0	0.0302	No match
CAAATCCTGT	5	0	0.0302	No match
CCAACAACCTG	5	0	0.0302	No match
CCCAGGCTGA	5	0	0.0302	ESTS
CCCTTGAGTC	5	0	0.0302	No match
CCTTCAAAA	5	0	0.0302	General transcription factor II I
CCTTCAAAC	5	0	0.0302	ESTS, highly similar to similar to nuclear domain 10 protein NDP52 (<i>H. sapiens</i>)
CTGCATTAAG	5	0	0.0302	ESTS, highly similar to enoyl-CoA hydratase (<i>H. sapiens</i>)
CTTGCAATCT	5	0	0.0302	ESTS, weakly similar to HORMONE SENSITIVE LIPASE (<i>M. musculus</i>)
CTTTGGGGGC	5	0	0.0302	ESTS
CTTTTCAGCA	5	0	0.0302	No match
GAAAGGTCAG	5	0	0.0302	ESTS
GAAACAGGACC	5	0	0.0302	ESTS
GACAAAAAAA	5	0	0.0302	ESTS
GATAATGCAC	5	0	0.0302	NADH dehydrogenase (ubiquinone) Fe-S protein 4 (18 kD)
GCACACACCC	5	0	0.0302	EST
GCCTGTCTTC	5	0	0.0302	ESTS
GGAATTTTTA	5	0	0.0302	ESTS
GGGGCTATCG	5	0	0.0302	No match
GGGGGCGCGC	5	0	0.0302	No match
GTGTAGACCA	5	0	0.0302	ESTS
TAGTGTTTTC	5	0	0.0302	No match
TCAAACCTGCC	5	0	0.0302	No match
TCATCTTTAA	5	0	0.0302	Calreticulin
TCCTCACGTG	5	0	0.0302	Pyruvate dehydrogenase 2
TCTTTAATCC	5	0	0.0302	ESTS
AAGCCACAGA	6	0	0.0147	ESTS, moderately similar to PUTATIVE SURFACE GLYCOPROTEIN C21ORF1 PRECURSOR (<i>H. sapiens</i>)
AGCAGCCCCT	6	0	0.0147	No match
CACTGGGTTG	6	0	0.0147	ESTS, highly similar to unknown (<i>H. sapiens</i>)
CGGACGGCTT	6	0	0.0147	No match
GGGATCCAG	6	0	0.0147	No match
GGTCTGCCC	6	0	0.0147	ESTS
TAGTGGCGTA	6	0	0.0147	α -internexin neuronal intermediate filament protein
TGAGCCTGGT	6	0	0.0147	Latent transforming growth factor β binding protein 3
TGGAGAACTA	6	0	0.0147	ESTS
TGGCCAAGCC	6	0	0.0147	Polyhomeotic (mPh2) mRNA
TGTTCTTCCC	6	0	0.0147	No match
TTTGTGACTG	6	0	0.0147	C-terminal-binding protein 1
GCCTTTATGA	12	27	0.0155	Ribosomal protein S24
TCCGGGCGAG	12	3	0.0163	Replication-dependent histone H2A.1 gene
GAACTTGCAA	18	7	0.0175	ESTS, moderately similar to FARNESYL PYROPHOSPHATE SYNTHETASE (<i>R. norvegicus</i>)
CGTCTGTGAA	10	2	0.0175	No match
GGCCCCAAAC	10	2	0.0175	V-crk-associated tyrosine kinase substrate

Table 3. (Continued)

TAG sequence	Ts65Dn	Normal	P	GenBank
AACGTTGGAA	0	6	0.0183	ESTs, weakly similar to cytosolic inorganic pyrophosphatase (<i>H. sapiens</i>)
AACTTGGCTG	0	6	0.0183	Guanylate kinase membrane-associated inverted 1
AAGCTGCCCT	0	6	0.0183	ORNITHINE AMINOTRANSFERASE PRECURSOR
AGCCCATCAA	0	6	0.0183	ESTs
AGGAGGGGTA	0	6	0.0183	RAB24, member RAS oncogene family
ATTCTGGCAG	0	6	0.0183	ESTs, highly similar to UBIQUITIN-CONJUGATING ENZYME E2-17 KD 3 (<i>H. sapiens</i> ; <i>R. norvegicus</i>)
CCGAATTACC	0	6	0.0183	Cornichon homolog (<i>Drosophila</i>)
CCTGTAATCC	0	6	0.0183	ESTs
CGGCCTACGT	0	6	0.0183	Superoxide dismutase 2, mitochondrial
GGCAGACTGT	0	6	0.0183	EST
GGGATCCAGG	0	6	0.0183	ESTs, weakly similar to coded for by <i>C. elegans</i> cDNA yk157f8.5
GGTAAAGTTA	0	6	0.0183	ESTs, weakly similar to MITOCHONDRIAL ELONGATION FACTOR TS PRECURSOR (<i>H. sapiens</i>)
GTCTTTTTTC	0	6	0.0183	ESTs, highly similar to ring-box protein 1 (<i>M. musculus</i>)
GTGGGGCGGG	0	6	0.0183	ESTs
TAACAAAGGA	0	6	0.0183	ESTS
TACCCCGTAC	0	6	0.0183	No match
TCCCCGTATA	0	6	0.0183	No match
TCTGGCAAAG	0	6	0.0183	EST
GCACCGAACA	28	14	0.0190	sid23p
GAGATGAGC	36	20	0.0192	RAB3A, member RAS oncogene family
CAAAAATAAA	25	12	0.0197	ESTs
GAGATGCCTG	10	23	0.0205	ESTs, highly similar to NADH-UBIQUINONE OXIDOREDUCTASE 42 kD SUBUNIT PRECURSOR (<i>B. taurus</i>)
ACGTCGTGCG	8	1	0.0206	No match
CCTTTCTGTC	8	1	0.0206	No match
CTTTGGGGAC	8	1	0.0206	ESTs, Highly similar to Down syndrome candidate region 1 (<i>H. sapiens</i>)
GCAGGCTTCG	8	1	0.0206	ESTs, weakly similar to HYPOTHETICAL 54.9 kD PROTEIN C02F5.7 IN CHROMOSOME III (<i>C. elegans</i>)
GCCTGCCCCG	8	1	0.02060	cdc37 homolog
GGGCCCCGGG	8	1	0.0206	Multiple matches
AAGAGAACAC	2	10	0.0208	No match
GGAGAAGAGC	2	10	0.0208	No match
TACGGCTAAA	2	10	0.0208	No match
AGAAAAAAAA	3	12	0.0209	Multiple matches
GAGTGCCTGT	3	12	0.0209	ESTs
TGACAGCTTG	5	0	0.0302	ESTs
TGAGCCAACT	5	0	0.0302	ESTs
TGAGTTACAG	5	0	0.0302	Nucleosome assembly protein 1-like 2
TGTGAGGCTC	5	0	0.0302	No match
TTCAGCTCGA	33	19	0.0303	Ribosomal protein S7
CCGCCAAGGG	9	2	0.0320	ESTs, moderately similar to hTOM34p (<i>H. sapiens</i>)
GCAGGTCCCC	9	2	0.0320	ESTs
GGGCCCAACT	9	2	0.0320	EST
GGGGAGACGC	9	2	0.0320	Fibroblast growth factor receptor 1
TGCAGTGCAC	9	2	0.0320	ESTs
CTGCGAGATT	25	13	0.0322	ESTs, highly similar to NADH-UBIQUINONE DEHYDROGENASE 24-kD SUBUNIT PRECURSOR (<i>R. norvegicus</i>)
AGAACTTCAA	7	1	0.0327	No match
CAGGGCGAGA	7	1	0.0327	ESTs, highly similar to hypothetical protein (<i>H. sapiens</i>)
CTGGGCTTGA	7	1	0.0327	No match
GCAAGAGAGA	7	1	0.0327	Glutamine synthetase
GCCCTGGCCC	7	1	0.0327	No match
GGATCCCCAA	7	1	0.0327	ESTs
TCCAACCTCT	7	1	0.0327	ESTs, weakly similar to weak similarity to <i>Bacillus stearothermophilus</i> . 30S ribosomal protein S21 (<i>C. elegans</i>)
TGAACCCATT	7	1	0.0327	Cytochrome oxidase, subunit VIa, polypeptide 1
TGGAGGAGCT	7	1	0.0327	ESTs, highly similar to formin-binding protein 11 (<i>M. musculus</i>)
GAGACCATCG	3	11	0.0333	ESTs, highly similar to (define not available 6013323< <i>M. musculus</i>)
GCGAGGAAGA	3	11	0.0333	No match
GGGGAAATCG	80	59	0.0341	ESTs, Moderately similar to THYMOSIN β -10 (<i>H. sapiens</i> ; <i>R. norvegicus</i> ; <i>Equus caballus</i>)

Table 3. (Continued)

TAG sequence	Ts65Dn	Normal	P	GenBank
CCAGGCCGGG	15	6	0.0347	Protein kinase C, ζ
GAGCCCCTTG	15	6	0.0347	No match
TGGAATGCAG	15	6	0.0347	Calnexin
AGTTGGAAC	12	4	0.0356	Nuclear factor of activated T-cells, cytoplasmic 2
CCCGTAGCCC	12	4	0.0356	Tropomyosin 2, β
GCGCCCTGAT	12	4	0.0356	No match
GGCACCATCT	12	4	0.0356	No match
TACCCAGGG	12	4	0.0356	No match
TTGATCCCCA	12	4	0.0356	Lysosomal membrane glycoprotein 1
GGGGCAGCCC	36	22	0.0357	Cornichon <i>Drosophila</i> -like
GCACAACTTG	213	181	0.0361	Multiple matches
CTACTGACAT	2	9	0.0364	No match
GCGAGGGAGC	2	9	0.0364	Dim1 (<i>S. pombe</i>)
GGAGCGCTAT	2	9	0.0364	ESTs
AAACATTGGG	0	5	0.0366	ESTs, weakly similar to Similar to <i>S. cerevisiae</i> EMP70 protein precursor (<i>H. sapiens</i>)
ACAACAAAAT	0	5	0.0366	ESTs
ACCCGCACCC	0	5	0.0366	No match
ACTCTTTCCT	0	5	0.0366	ESTs
ACTGTGTCTT	0	5	0.0366	ESTs
AGAGGCACTT	0	5	0.0366	ESTs, highly similar to ACETYL-COENZYME A SYNTHETASE (<i>Escherichia coli</i>)
AGATGGCTTT	0	5	0.0366	Mast cell-growth factor
AGCCGTGTAT	0	5	0.0366	ESTs
AGGCTGGTGA	0	5	0.0366	Proteasome (prosome, macropain) subunit, β type 1
CAAATGGACT	0	5	0.0366	ESTs
CACAGCGTTG	0	5	0.0366	No match
CACTTTTTTT	0	5	0.0366	No match
CCAAGGCTGC	0	5	0.0366	ESTs
CGGGACCCCA	0	5	0.0366	No match
CTAGTCGCAA	0	5	0.0366	ESTs, highly similar to THYMIDINE PHOSPHORYLASE (<i>H. sapiens</i>)
CTGTGTAATT	0	5	0.0366	ESTs
GAAACCAAGGA	0	5	0.0366	ESTs, weakly similar to PLATELET-ENDOTHELIAL TETRASPAN ANTIGEN 3 (<i>M. musculus</i>)
GAATTGATGA	0	5	0.0366	Secretogranin II
GATCTGACCC	0	5	0.0366	ESTs
GCCACGCCCT	0	5	0.0366	No match
GCTTAAAAAT	0	5	0.0366	ESTs
GGAAGTTGGC	0	5	0.0366	No match
GGGAAGAGAT	0	5	0.0366	ESTs
GGTACCCCCA	0	5	0.0366	ESTs
GTTTTCTAGT	0	5	0.0366	ESTs, highly similar to PRE-mRNA SPLICING HELICASE BRR2 (<i>S. cerevisiae</i>)
TAGACCAGGC	0	5	0.0366	ESTs, weakly similar to VITAMIN K-DEPENDENT γ -CARBOXYLASE (<i>H. sapiens</i>)
TATGTCAAGT	0	5	0.0366	No match
TCCAGAAAAT	0	5	0.0366	No match
TCCCCGTTCA	0	5	0.0366	No match
TGAACTGTGT	0	5	0.0366	ESTs
TGGATGCTGG	0	5	0.0366	Adenomatous polyposis coli binding protein Eb1
TGGGCCACAC	0	5	0.0366	ESTs, weakly similar to KIAA0990 protein (<i>H. sapiens</i>)
TTCCCCACGT	0	5	0.0366	Cell division cycle 2 homolog <i>S. pombe</i> -like 2
CAATAAACTG	23	12	0.0367	Suppressor of initiator codon mutations-yeast homolog-related sequence 1
GGCTGGGGGC	26	14	0.0369	Profilin 1
ACCGGTATTT	5	14	0.0376	Protein kinase C, β
CCAATGCAGC	5	14	0.0376	ESTs, highly similar to PROBABLE 3-OXOADIPATE COA-TRANSFERASE SUBUNIT B (<i>Bacillus subtilis</i>)
AAAGTGTTTC	1	7	0.0379	ESTs
ATCTCAAACC	1	7	0.0379	ESTs
CCTACAGTCC	1	7	0.0379	No match
CGCAATTCAA	1	7	0.0379	No match
GAGAAAGTTC	1	7	0.0379	ESTs
GGATGATGGT	1	7	0.0379	ESTs, highly similar to EXTRACELLULAR RIBONUCLEASE LE PRECURSOR (<i>Lycopersicon esculentum</i>)

Table 3. (Continued)

TAG sequence	Ts65Dn	Normal	P	GenBank
GT CAGGGGGA	1	7	0.0379	ESTs
TCCCCGTCCA	1	7	0.0379	No match
TCCCTACTAA	1	7	0.0379	No match
TGAGCAGTGG	1	7	0.0379	No match
TGTGTTCCCA	1	7	0.0379	ESTs, weakly similar to (define not available 5931573, <i>M. musculus</i>)
GGATTTGGCT	45	66	0.0388	Keratin complex 2, basic, gene1
ATGAAACTTT	19	9	0.0393	ESTs
T CAGAGGCCT	22	37	0.0396	ESTs
AACAAAGCAA	10	3	0.0402	ESTs, moderately similar to unknown (<i>H. sapiens</i>)
AGGCCAGCTG	10	3	0.0402	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F
GCCAAATTTG	10	3	0.0402	ESTs, highly similar to 26S proteasome subunit p112 (<i>R. norvegicus</i>)
GGGCTGGAAT	10	3	0.0402	ESTs
CTTGCAAGTG	13	25	0.0417	ESTs, highly similar to NADH-UBIQUINONE OXIDOREDUCTASE B22 SUBUNIT (<i>B. taurus</i>)
GAGTTCCTG	13	25	0.0417	ESTs, moderately similar to HSPC035 protein (<i>H. sapiens</i>)
GCTGGCAGCC	32	19	0.0417	RAD9 homolog (<i>S. pombe</i>)
TTGGCTGCCC	43	28	0.0422	Ribosomal protein S14
GTCTGCTGAT	6	15	0.0439	Guanine nucleotide-binding protein, β -2, related sequence 1
GAGGAGTGGG	9	19	0.0461	NO match
CTTCTCATTT	3	10	0.0462	Membrane transporter protein
GGCAATCAA	3	10	0.0462	No match
TGTCGCCAGG	3	10	0.0462	No match
CCAGAACAGA	14	26	0.0464	Ribosomal protein L30
GCCTGTGGTG	21	11	0.0469	Lymphocyte antigen 6 complex, locus H
GGGTGCA GCC	25	14	0.0470	FK506-binding protein 8 (38 kD)
GTGCCCTCCA	34	21	0.047	No match
TGGAACCTTG	13	5	0.0478	ESTs
TTCTCCTCAG	13	5	0.0478	ESTs, moderately similar to (define not available 5815343, <i>M. musculus</i>)
TATGCAACCC	33	21	0.0486	Necdin
AAAA CAGTGG	15	27	0.0489	Ribosomal protein L37a
GGGAGGGAGG	17	8	0.0499	No match
AGCAGTCCCT	11	4	0.0542	Homogentisate 1,2-dioxygenase
CTCAGAAAGC	11	4	0.0502	ATPase, H ⁺ transporting, lysosomal (vacuolar proton pump), α 70 kD, isoform 2
GCTGCCTTCC	11	4	0.0502	Harvey rat sarcoma virus oncogene
TGAACCTGAG	11	4	0.0502	ESTs
CTCCTGCAGC	5	13	0.0508	sid478p
GACGAGATTC	5	13	0.058	ESTs, weakly similar to Lpe5p (<i>S. cerevisiae</i>)

For each tag (tag sequence) the number of its copies in Ts65Dn library (Ts65Dn) and normal library (Normal) together with the estimated p-chance and GenBank match (GenBank match) are shown. Complete table available as online supplementary material at www.genome.org. An ongoing update of the table is available at medger.unige.ch/research/projects.html.

(Reeves et al. 1995). This should theoretically lead to overexpression ($1.5 \times$ the normal level) of the genes from this part of the chromosome. We have examined the expression of the 24 MMU16 genes known from the region between App and Mx1 as represented by their corresponding tags (Table 5). We have detected at least one tag for 15 of these genes; however, only three, *Ifnar2* (three tags in trisomy and none in normal SAGE library), *Ifngr2* (three tags in trisomy and none in normal SAGE library), and *Cbr* (nine tags in trisomy and three tags in normal SAGE library), showed overexpression in the Ts65Dn male versus the normal male SAGE library. Interestingly, the level of expression of most known triplicated genes is too low to achieve statistical significance.

DISCUSSION

Understanding not only the spatial and temporal expression patterns of individual genes but also how these relate to expression patterns of other genes and to events such as changes in behavioral state, onset of disease, and response to drugs, is of great interest and can provide valuable insights into their molecular physiology. To study the effects of aneuploidy in the brain transcriptome we have sequenced ~150,000 tags, 50,000 each from normal male, Ts65Dn male, and normal female P30 brain SAGE libraries. Out of ~150,000 sequenced tags we have observed 45,856 unique tags. To estimate how many different unique transcripts these 45,856 tags represent, we used a computed sequencing error of 6.8% (Velculescu et al. 1997) and the

Table 4. List of Tags Corresponding to Ribosomal Protein Genes with Statistically Significant Differences between Normal and Ts65Dn Male Brain SAGE Library

Description	TAG	Normal males	Trisomic males	P	Normal females	3T3 (Nor50K)
Ribosomal protein L26	TGGGCATCCA	41	12	0.0000	26	0
Ribosomal protein L5	CTGCTATCCG	32	9	0.0003	19	40
Ribosomal protein S8	TGTAGTGTA	0	8	0.0035	6	12
Ribosomal protein S6	GCAGAGTGCG	14	32	0.0044	19	85
Ribosomal protein S3	CCCCAGCCAG	48	25	0.0065	42	156
Ribosomal protein S19	ACCAAGATCT	13	3	0.0112	17	46
ESTs, highly similar to 60S RIBOSOMAL PROTEIN L37 (<i>Homo sapiens</i> ; <i>Rattus norvegicus</i>)	GATTCCGTGA	64	39	0.0113	67	108
Ribosomal protein S24	GCCTTTATGA	27	12	0.0155	30	43
Ribosomal protein S7	TTCAGCTCGA	19	33	0.0303	38	44
ESTs, weakly similar to <i>Bacillus stearothermophilus</i> 30S ribosomal protein S21 (<i>Caenorhabditis elegans</i>)	TCCAACCTCCT	1	7	0.0327	7	2
EST, weakly similar to 60S ACIDIC RIBOSOMAL PROTEIN P2 (<i>Rattus norvegicus</i>)	GGATTTGGCT	66	45	0.0388	78	44
Ribosomal protein S14	TTGGCTGCCC	28	43	0.0422	40	73
Ribosomal protein L30	CCAGAACAGA	26	14	0.0464	23	68
Ribosomal protein L37a	AAAACAGTGF	27	15	0.0489	35	36

The identity for each tag (Description) and statistical significance (P) of observed differences between normal and Ts65Dn SAGE libraries are presented. The number of tags in SAGE libraries derived from normal (Normal males) or Ts65Dn (Trisomic males) mouse male brains, normal female brains (Normal females), and mouse embryonic cell line 3T3 are shown. Tags from the 3T3 SAGE library were normalized for a total of 50,000.

calculation that each transcript is represented by ~1.6 SAGE tags (Velculescu et al. 1999). These assumptions lead to an estimated number of 26,711 detected genes. Because we do not reach the plateau in unique tag discovery (Fig. 1), the total number of genes expressed in mice brain is higher but probably is ~30,000–35,000. Comparison of the data between the human and

mouse transcriptome shows a similar percentage of cellular transcripts in the various classes of abundance of unique tags (Table 1). That many more unique tags were detected in the category of five or fewer copies per cell in humans (37,293 vs. 127,342 tags in the mouse brain and the human transcriptome, respectively) reflects the total number of tags sequenced (3.5 million

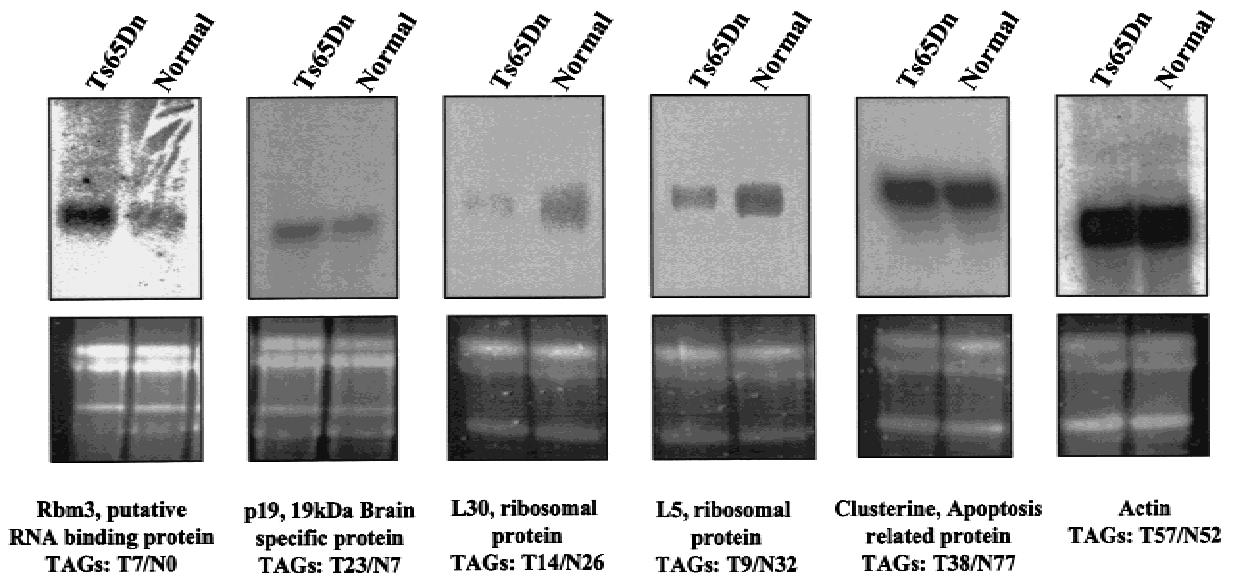


Figure 4 Northern blot analysis of some examples of differentially expressed genes between normal and Ts65Dn mouse serial analysis of gene expression (SAGE) libraries. Total RNA isolated from brain of four control males and four Ts65Dn male mice was blotted and hybridized with probes detecting Rbm3, p19, L30, and L5 ribosomal protein genes, clusterin, and actin transcripts. The top panel shows the results of autoradiography, the bottom panel shows the corresponding agarose gels prior to blotting (the two bands represent the 28S and 18S rRNAs). The number of tags detected in normal (N) and Ts65Dn (T) male SAGE libraries are also shown.

Table 5. List of Known MMU16 Genes Present in Three Copies in Ts65Dn Mice and Their Expression Level as Detected by the Number of Corresponding Tags in Normal and Ts65Dn SAGE

Gene name	GenBank No.	pA site	TAG sequence	Ts65Dn Normal	
<i>App</i>	X59379	yes	A T A G C T T T C T	26	23
<i>Grik1</i>	X66118	yes	G A T A G A G G G A	0	0
<i>Il10rb</i>	U53696	no	A A C A T T T T G G	0	0
<i>Sod1</i>	M35725	yes	A G A A A C A G A	7	10
<i>Tiam1</i>	U05245	yes	A T G T G T C T A T	1	0
<i>Cbfa2</i>	D13802	yes	C A C A G A C C C C	0	0
<i>Mak-V</i>	AF055919	no	A G T A A G G A C T	0	1
<i>Synp</i>			No mouse homolog		
<i>Rack17</i>			No mouse homolog		
<i>lfnar2</i>	AF013486	yes	G A G A G C T A C G	3	0
<i>lfngt1</i>			No mouse homolog		
<i>C21orf4</i>			No mouse homolog		
<i>Gart</i>	U01024	yes	G C C A A A C T G A	1	1
<i>Son</i>	AF1935606	yes	T G T A T T A C A G	1	0
<i>Itsn Esi1</i>	AF132478	no	G A C C C C A G C C	0	0
<i>Itsn Esi1L</i>	AF132481	no	A G G T C C C C A C	0	0
<i>Atp5o</i>			No mouse homolog		
<i>Slc5a3</i>	NM_017391	no	T T T G T T T A T T	0	0
<i>Mirp1</i>			No mouse homolog		
<i>Kcnq1</i>	U70068	yes	T G C A G C C T G C	0	1
<i>Dscr1</i>			No mouse homolog		
<i>Clic3</i>			No mouse homolog		
<i>lfngr2</i>	U69599	yes	C C T T T A T T C C	3	0
<i>Cbr</i>	U31966	yes	G T G A A C C C A A	9	3
<i>Sim2</i>	D64135	yes	T T G T G T G T A A	0	0
<i>Ttc3-Tprd</i>	AB008516	yes	C C T T A C A C T T	4	4
<i>Dyrk1a</i>	U58497	no	G T C G A C G C G G	0	0
<i>Kcnj6</i>	U51122	yes	T G T G A G A T G C	0	0
<i>Erg</i>	S66169	no	G G C T C C T A T C	0	0
<i>Ets2</i>	J04103	no	C C A T C C T G G G	0	2
<i>Hmg14</i>	X53476	yes	T A A T A A A A T	1	0
<i>Pcp4</i>	X17320	yes	A A G A G A A A C C	46	39
<i>Dscam</i>			No mouse homolog		
<i>Mx1</i>	M12279	yes	T G T T T G C T T C	1	0
<i>Mx2</i>	J03368	yes	C T C C A G C T C C	0	0

For each gene (Gene name, GenBank no.) the corresponding most 3' tag was used to determine the tag frequency in SAGE libraries derived from normal or Ts65Dn mouse male brains.

tags from 19 normal and diseased tissue types in humans). Seventy-six percent of the mouse brain tags had no matches in the public databases, compared with 46% in the human transcriptome (Velculescu et al. 1999). Unmatched tags in both cases are mainly among the genes with low expression levels and this difference is likely to be caused by the lower number of mRNAs and ESTs described in mice as compared with humans. As expected, highly expressed tissue-specific transcripts that have been detected in other SAGE studies (e.g., trypsin in pancreas [Velculescu et al. 1995] or desmin and troponin in muscle [Welle et al. 1999]) are expressed at low levels or not at all in mouse brain (trypsin, no tags; desmin, no tags; troponin, three tags). A comparison of the 15 most abundant mouse brain transcripts with data from the human brain (Table 2; Velculescu et al. 1999) revealed that, on the whole, these genes are expressed at comparable levels in both species. However, these genes were expressed

at slightly higher levels in mouse brain compared with human brain, which may reflect structural differences between mouse and human brain or differences in age. More systematic comparisons between mouse and human transcriptomes will be important in establishing the mouse as a valid model for expression studies of human development, disease, and therapies. Improved bioinformatics tools and standard gene nomenclature between humans and mouse could improve and facilitate such cross-species comparisons.

That SAGE is an accurate reflection of the content of the RNA used in the experiment has been established by many studies (Hermeking et al. 1997; Madden et al. 1997; Polyak et al. 1997; Velculescu et al. 1997; Zhang et al. 1997; Chen et al. 1998; Gonzalez-Zulueta et al. 1998; He et al. 1998; Hibi et al. 1998; Hashimoto et al. 1999; Kal et al. 1999; van den Berg et al. 1999; Welle et al. 1999). An internal control of a differential gene expression study by SAGE is that the

expression levels of the majority of transcripts remain the same between the compared samples. Two additional controls of the quality of our data have been performed. We have checked the completeness of *Nla*III digestion by analyzing the occurrence of tags derived from internal *Nla*III sites in several genes including β -actin (GenBank accession no. X03672), α -actin (GenBank accession no: X03766) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; GenBank accession no: M32599). For all of these genes, there were only between one and four tags matching possible results of partial digestion by *Nla*III, compared with 68 tags derived from the most 3' *Nla*III site of α -actin, 167 of β -actin, and 490 of GAPDH. Thus the *Nla*III digestion in the experiments described here went almost to completion. Biological and statistical (as opposed to statistical only) significance of observed differences can be added by confirming their presence in independent biological samples. Northern blot analyses in independent brain RNA samples from normal and Ts65Dn mice confirmed the differential expression of *Rbm3* (seven tags in trisomy and none in normal SAGE library), *p19* (23 tags in trisomy and seven tags in normal SAGE library), *L30* (14 tags in trisomy and 26 tags in normal SAGE library), and *L5* (nine tags in trisomy and 32 tags in normal SAGE library) ribosomal protein genes and cytoskeletal actin (57 TAGs in trisomy and 52 tags in normal SAGE library). The observed underexpression of clusterin in the brains of Ts65Dn mice could not be confirmed. RNA expression differences observed using SAGE tags may not be confirmed because of incorrect identification of the transcript, different samples used for collection and confirmation of the data, and variability of Ts65Dn phenotypes possibly because of the fact that the Ts65Dn mice are not inbred, because they originated from B6 and C3H backgrounds.

The level of expression of most known genes from the triplicated region of mouse MMU16 in Ts65Dn is too low to observe statistically significant differences. Of 15 genes for which at least one tag was detected in the 100,000 sequenced from normal and Ts65Dn male SAGE libraries, only three (*Ifnar2*, *Ifngr2*, and *Cbr*) are overexpressed (not statistically significant) in Ts65Dn mice (Table 5). Overexpression of two MMU16 genes, *App* and *Sod1*, in Ts65Dn has previously been documented (Reeves et al. 1995; Holtzman et al. 1996); however, at the depth of our tag collection we did not observe these results in our SAGE analysis (for *App*, 26 tags in trisomy and 23 tags in the normal SAGE library were overexpressed; for *Sod1*, seven tags in trisomy and 23 tags in the normal SAGE library were overexpressed).

Our data suggest that trisomy for the part of MMU16 present in Ts65Dn mice results in limited changes in the transcription level of many genes (330

changes with $P < 0.05$), rather than in the induction of strong overexpression or repression of a few transcripts. We have obtained similar results analyzing gene expression in T21 versus normal amniocytes (Scott et al., unpubl.) and protein expression (~2000–2500 proteins analyzed) in brains of Ts65Dn and normal males using 2D gels (Chrast et al., unpubl.). It is evident that the SAGE analysis, other differential gene expression techniques, and 2D protein gels are currently capable of being quantitative only for abundant transcripts and proteins. It is also difficult to assign what differences are physiologically significant. Thus differences in low-abundance mRNAs, small physiologically significant differences, and differences specific to temporal and spatial gene expression would not have been detected in this study.

Apoptosis was described to accelerate neuronal death in cultured neurons from human DS brains (Busciglio and Yanker 1995) and T16 mouse model of DS (Bambrick and Krueger 1999). However, our analysis revealed only one differentially expressed gene related to apoptosis, the clusterin (38 tags in trisomy and 77 tags in normal SAGE library), and this difference was not confirmed by Northern blot.

Grouping genes according to function to detect trends in the expression of identified transcripts (with or without significant differential expression) revealed that there are 14 ribosomal protein genes (nine of which are underexpressed) that show statistically significant difference in expression (Table 4). No other class of genes is significantly overrepresented. The mammalian ribosome is composed of four RNA species and ~80 different proteins that constitute ~80% of all cellular RNA and 5%–10% of cellular protein (Wool 1979). There has been a report of reduced activity of ribosomal genes with age in DS (Borsatto and Smith 1996). There are two additional lines of evidence that suggest that the underexpression of ribosomal proteins may be important in development and maintenance of DS/Ts65Dn phenotypes. First, the ribosomal protein genes are not underexpressed in normal P30 female brain and 3T3 cells (Table 4). Second, a similar finding was observed in a comparison of gene expression between T21 and normal human amniocytes by SAGE, including the underexpression of three of the same ribosomal proteins (Scott et al., unpubl.).

In *Drosophila*, several *Minute* loci (reduced body size, diminished fertility, and short, thin bristles) have been characterized and all have been found to encode ribosomal proteins (Kongsuwan et al. 1985; Hart et al. 1993; Andersson et al. 1994; Cramton and Laski 1994; Schmidt et al. 1996; Saeboe-Larsen et al. 1998). *Minute* cells probably contain fewer ribosomes and thus have a reduced capacity for protein synthesis. Findings consistent with reduced expression of ribosomal protein S4 are implicated in certain features of Turner syn-

drome (45, X; Fisher et al. 1990; Watanabe et al. 1993; Zinn et al. 1994). It has been predicted that ribosomal protein deficiencies contribute to the abnormal development and poor viability of monosomic human fetuses (Kenmochi et al. 1998).

Mutations of the ribosomal protein S19 (*RPS19*) gene, which is underexpressed in both this study (three tags in trisomy and 13 tags in normal SAGE library) and the study of normal human versus T21 fetal fibroblasts, were recently identified in patients with Diamond-Blackfan anemia (DBA; Draptchinskaia et al. 1999). DBA or congenital pure red blood cell aplasia is variably associated with a wide range of abnormalities, some of which are similar to those of DS. These include short stature, craniofacial and thumb abnormalities, cardiac (atrial or ventricular septal defects) and urogenital malformations, epicanthal folds, cataracts, prenatal or postnatal growth retardation, and a moderately increased risk of developing hematological malignancies. A wide range of clinical presentations, and even clinically silent effects, are associated in DBA with mutations in the *RPS19* gene, even for identical mutations within the same family.

Although variable reduction in the rate of protein synthesis could contribute to and modulate expression of DS/Ts65Dn phenotypes, some ribosomal proteins have been shown to have extraribosomal functions, such as rat and *Drosophila melanogaster* ribosomal protein S3, which also functions as a DNA-repair endonuclease (Wilson et al. 1994). S3 is also underexpressed in both this study (N48/T25) and in our human fibroblast SAGE study.

In conclusion, our study contributes to the establishment of a mouse brain transcriptome, an important step that enables analyses of gene expression induced by disease states and other biological conditions. P30 mice are just before sexual maturity (~6 wk old); therefore our data may also serve as a starting point to determine differences in gene expression between male and female brains induced by hormonal and other changes that occur with sexual maturation. The comparison of normal and Ts65Dn male brain SAGE libraries revealed 330 differentially expressed transcripts. Identification of all transcripts corresponding to these TAGs will enable the construction of microarrays and a higher throughput analysis of their expression in additional tissues and developmental stages from mouse models of DS. Use of samples from Ts65Dn and other mice overexpressing single transgenes from the so-called DS-critical region may also help to dissect the contributions of distinct chromosomal regions and individual genes to the expression profile. We anticipate that with a better understanding of the molecular pathophysiology of DS new therapeutic approaches may be developed to this complex disorder.

METHODS

Tissue Collection, Diagnosis, and RNA Isolation

Whole brains and spleens from four control males, three control females, and three Ts65Dn P30 mice were collected and stored in liquid nitrogen. Ts65Dn mice are not inbred but are maintained by mating female carriers of the 17¹⁶ chromosome (B6C3H-Ts65Dn) to B6EiC3HF1 hybrid male mice obtained from crossing of C57Bl/6Jei × C3H/HeSnJ strains. The phenotype is already partially established in P30 Ts65Dn mice. The comparison of gene expression pattern at this age may therefore reveal genes that are involved in maintenance, rather than the development, of phenotypes. Ts65Dn mice have only mild behavioral changes that are difficult to detect by simple observation of the animals (M.T. Davisson, C. Schmidt, pers. comm.). Thus, the presence of the partial trisomy was diagnosed by fluorescence in situ hybridization analysis. Interphase nuclei of spleen cells (a detailed protocol can be found at <http://www.biomednet.com/home>) were hybridized using a biotin-labeled MMU16-specific probe (Applied Genetics Laboratories) mapping distal to App in the region that is triplicated in Ts65Dn mice. Littermate mice that did not have partial trisomy 16 were used as controls. Poly A⁺ RNA was prepared from each mouse brain separately using the FastTrack 2.0 Kit from Invitrogen. mRNA was quantified by spectrophotometry and equal amounts of mRNA from each brain were used to make three pools for SAGE library construction (Ts65Dn males, normal males, and normal females).

Generation of SAGE Libraries

SAGE libraries were produced following the SAGE protocol (Velculescu et al. 1995), available at http://www.sagenet.org/sage_protocol.htm: 5 µg of poly A⁺ mRNA were converted to cDNA (GibcoBRL) using a gel-purified 5'-biotinylated oligo-(dT)₁₈ (Integrated DNA Technologies, Inc.). cDNA was digested with NlaIII (NEB), the anchoring enzyme, and 3' cDNAs were isolated using streptavidin magnetic beads (DynaM-280). 3' cDNAs were split into two pools, and SAGE linkers 1 and 2 (synthesized by Integrated DNA Technologies, Inc.) were ligated to pools 1 and 2, respectively. SAGE tags were released with the tagging enzyme BsmFI (NEB), blunt ended with the Klenow polymerase fragment, and the tags from pools 1 and 2 were ligated to each other over night at 16° C. A 1:250 dilution of the ligation product was amplified with 28 cycles of PCR (192 reactions). Precipitated PCR products were run on 12% polyacrylamide gel and only the 102-bp band containing ditags (a combination of two SAGE tags in a tail-to-tail orientation) was isolated and digested with NlaIII. The products of the digestion were run on a 12% polyacrylamide gel and the 24–26-bp bands containing ditags were purified and used for self-ligation. Concatamers were run on an 8% polyacrylamide gel and a fraction from 700 bp to 1500 bp was isolated and cloned into pZERO vector (Invitrogen) digested with SphI. Cloned concatamers were amplified by colony PCR using T7 and SP6 primers. PCR products were purified using QIAquick 96 PCR purification kits (QIAGEN) and used as templates for sequencing with BigDye terminators using standard protocols for the ABI377 automated sequencer.

SAGE Data Analysis

SAGE data are available as SAGE projects and Microsoft Access databases at <http://medgen.unige.ch/research/projects.html>. Data analysis was performed using the SAGE analysis software

(version 3.03; <http://www.sagenet.org/>). The statistical approach to compare two SAGE libraries used by this software is based on Monte Carlo simulations. For each P -chance calculation, 100,000 simulations are performed assuming the null hypothesis (the level, kind, and distribution of transcripts is the same for the two populations being compared); the P -chance value represents the fraction of simulations that yielded a difference equal to or greater than the observed difference. This is a relative probability of obtaining the observed differences because of the random variation (Zhang et al. 1997). tags derived from linkers and duplicate ditags were excluded from the analyses. Transcript tags were matched to known genes and ESTs using SAGE tag to UniGene clusters developed by A. Lash (Lal et al. 1999). The analysis was done using the NCBI database (<ftp://ncbi.nlm.nih.gov/pub/alah/Mm/>), UniGene build 75, <http://www.ncbi.nlm.nih.gov/UniGene>), and the Microsoft Access software. As UniGene cluster numbers are not stable over time, the most recent tag to cluster mapping should be obtained (<ftp://ncbi.nlm.nih.gov/pub/alah/Mm/>). The mouse embryonic cell line (3T3) SAGE library (28,531 tags) was downloaded from <http://www.sagenet.org/SAGEData/sagedata.htm>. Human brain data from two SAGE libraries, BB542 (95,254 tags) and normal pool (61,218 tags), were downloaded from <http://www.ncbi.nlm.nih.gov/SAGE/sagelb.cgi>. Transcript abundance per cell was determined by dividing the observed number of tags for a given transcript by the total number of transcripts obtained. An estimate of 300,000 transcripts per cell was used to convert the observed tags to copies per cell.

Northern Blot Analysis

Fifteen micrograms of total RNA isolated from the brains of four control males and four Ts65Dn male mice were electrophoresed on agarose-formaldehyde gel, blotted onto Gene-Screen Plus membrane (NEN Research Products), and hybridized as described (Sambrook et al. 1989). We have used the following RT-PCR probes prepared from normal mouse brain cDNA: Mouse skeletal actin (GenBank accession no. M12866), 155 bp between oligonucleotides mactE6F 5'GCTGACCG CATGCAGAAGGA3' and mactE7R 5'CTCCTGCTTGGTGA TCCACA3'; mouse putative RNA-binding protein 3 (GenBank accession no. AB016424), 314 bp between RBM3F 5'AGGC ACTCAGGATTATTGTT3' and RBM3R 5'TAGCTCCATAAAG GACATCC3'; Mouse p19 protein (GenBank accession no. U17259), 367 bp between p19F 5'CATTCTCAACTAG GAATGC3' and p19R 5'GAATTCAATTGTCATTCCTG3'; mouse clusterin (GenBank accession no. L08235), 150 bp between clusterinF 5'CATCACCTTCCTATATGTAG3' and clusterinR 5'AGGCATTAGTGTACAGAATC3'; mouse ribosomal protein L5 (GenBank accession no. X83590), 205 bp between ribL5F 5'AGGAAGATGAAGATGCGTAT3' and ribL5R 5'GCAACCCGATCTTCTTCTG3'; and mouse ribosomal protein L30 (GenBank accession no. K02928), 190 bp between RibL30F 5'GATGATCAGACAAGGCAAAG3' and RibL30R 5'TGGGTCAATGATAGCCAGTG3'.

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