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Global up-regulation of chromosome 21 gene expression in the developing Down syndrome brain

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Abstract

Down syndrome (DS) results from complete or partial triplication of human chromosome 21. It is assumed that the neurological and other symptoms are caused by the overexpression of genes on chromosome 21, but this hypothesis has not yet been assessed on a chromosome-wide basis. Here we show that expression of genes localized to chromosome 21 is globally up-regulated in human fetal trisomy 21 cases, both in cerebral cortex extracts and in astrocytic cell lines cultured from cerebral cortex. This abnormal regulation of gene expression is specific to chromosome 21. Our data describe transcriptional changes that are specific to many genes assigned to chromosome 21 and do not directly measure the clinical phenotype of DS. However, it is possible that these gene expression changes ultimately relate to the phenotypic variability of DS.

Down syndrome (DS), with an incidence of 1 in 700–1000 live births, is the leading genetic cause of mental retardation and the most common autosomal aneuploidy compatible with postnatal survival. Several dozen phenotypic features of the disease with varying frequencies among affected individuals have been identified, including mental retardation, neuropathological modifications (in DS patients over 35 years of age) identical to those in the brains of individuals with Alzheimer disease, congenital heart defects, characteristic facial and skeletal features, and increased incidence of seizures [1,2]. The majority of these features do not occur in every individual with trisomy 21 (TS21), and those features that are present can vary considerably in severity [2]. Although the cause of DS has been known since 1959 [3,4], the mechanisms by which three copies of chromosome 21 result in the disruption of normal development are not well understood. There are currently two major hypotheses to account for the phenotype of DS. According to the "developmental instability" hypothesis, a global chromosome 21 dosage imbalance causes the varied phenotypic profile of DS [5,6]. An alternate hypothesis is that dosage imbalance of a small number of chromosome 21 genes in a critical region causes the DS phenotype [7]. The two hypotheses are not necessarily mutually exclusive. It is possible that single genes may be involved in specific DS phenotypes, while some other DS phenotypes may be due to disturbances in gene dosage balance as a result of the extra chromosomal material [8].

A fundamental question for either hypothesis is the extent to which genes, especially those assigned to chromosome 21, are differentially expressed in DS. The recent sequencing and annotation of chromosome 21 [9] allow us to evaluate this issue. Using microarray technology, we examined gene expression in the developing DS brain and observed a chromosome-wide increase in transcription levels for genes that have been assigned to chromosome 21.

Results

We employed two complementary strategies to assess gene expression in DS: analyses of frozen fetal brain samples and astrocyte cell lines derived from fetal brain. These

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Table 1
Samples used for DS studies

Sample[a]	Source[b]	Type	Age (gw)	Gender	Race	PMI (h)	Microarray[c]
700	Astrocyte	Control	17	Male	African American	1	U95Av2
907	Astrocyte	TS21	17	Male	Caucasian	3	U95Av2
696	Astrocyte	Control	17	Male	Caucasian	1	U95Av2
848	Astrocyte	TS21	21	Female	Caucasian	5	U95Av2
1521	Astrocyte	Control	18	Female	African American	1	U95Av2 U133A
1479	Astrocyte	Control	17	Female	African American	1	U95Av2 U133A
748	Astrocyte	TS21	20	Male	Caucasian	2	U95Av2 U133A
1478	Astrocyte	TS21	18	Female	Caucasian	4	U95Av2 U133A
1390	Cerebrum	Control	18	Female	African American	1	U133A
1411	Cerebrum	Control	18	Male	African American	1	U133A
1521	Cerebrum	Control	18	Female	African American	1	U133A
1565	Cerebrum	Control	18	Female	African American	2	U133A
847	Cerebrum	TS21	18	Female	Caucasian	1	U133A
1218	Cerebrum	TS21	19	Female	Caucasian	1	U133A
1389	Cerebrum	TS21	18	Male	Caucasian	1	U133A
1478	Cerebrum	TS21	18	Female	Caucasian	4	U133A

Abbreviations used: TS21, trisomy 21; gw, gestational weeks; PMI, postmortem interval.
[a] Samples are identifier numbers from the Brain and Tissue Bank for Developmental Disorders, University of Maryland, Baltimore.
[b] Astrocyte cell lines were derived from cerebral cortex.
[c] Affymetrix chips U95Av2 (representing 12,625 human gene probe sets) and/or U133A (representing 22,283 gene probe sets) were employed.

astrocytes represent a homogeneous source of material, in contrast to brain samples, which contain multiple cell types. Cell cultures or dissected tissue samples were obtained from sixteen 17- to 20-week-gestation fetal abortuses with a postmortem interval of 1–5 h (Table 1). The diagnosis of DS was confirmed in 8 samples by karyotype analysis, while 8 control samples were euploid. The astrocyte cell lines were characterized by immunocytochemistry for TS21 samples (Fig. 1) and euploid samples (data not shown) [10]. Both types of samples exhibited specific staining for the astrocyte marker glial fibrillary acidic protein (GFAP) (Figs. 1G and 1H). These cell lines were determined to have $<1\%$ contamination by neurons (based on MAP2 staining), 0% by microglia (based on CD68 staining), and ≤ 2–5% by fibroblasts (based on cellular fibronectin staining).

Total RNA was extracted and the expression levels of up to 18,462 transcripts, representing approximately 15,106 genes, were quantitated using microarrays. Data analysis resulted in the identification of genes regulated in DS brain or astrocytes relative to euploid controls. Based on an ANOVA, 679 individual genes were significantly regulated in TS21 astrocytes relative to euploid controls ($p < 0.05$) and 725 individual genes were significantly regulated in TS21 brain ($p < 0.05$). However, no individual gene was significantly regulated in TS21 relative to euploid samples when a conservative Bonferroni correction (α level = 0.05/number of observations) was applied.

We compared gene expression from each TS21 astrocyte cell line ($n = 4$) and each TS21 brain sample ($n = 4$) to an appropriate age-matched control and plotted the number of genes observed versus the distribution of local Z scores. The mean local Z scores were -0.02 to 0.01 for the eight comparisons. (Thus, for the set of all genes analyzed the mean ratio of gene expression values for TS21 relative to euploid samples was equal.) We then further annotated the gene expression data according to chromosome using the DRAGON database [11,12]. In all eight TS21 cases, density distribution plots revealed that genes localized to chromosome 21 had significantly different distributions of local Z scores relative to those of all other genes (i.e., genes not localized to chromosome 21 and genes not assigned to any chromosome), with a consistent up-regulation of expression of genes on chromosome 21 (Figs. 2A–2H). This increase was statistically significant based upon the results of χ^2 tests, with probability (p) values ranging from 8.99×10^{-107} to 2.95×10^{-9} for all of the four brain sample comparisons of TS21 to euploid brain samples and from 3.08×10^{-80} to 1.40×10^{-18} for astrocyte samples. The mean Z score in the eight plots (Figs. 2A–2H) ranged from 1.1 to 1.8 for genes localized to chromosome 21. This dramatic increase was specific to that chromosome; the distribution of gene expression values was not significantly different for any chromosome other than 21.

These observations were visualized using principal components analysis (PCA), a technique for the reduction of highly dimensional data into two- or three-dimensional space [13]. We performed PCA based on the distribution of local Z scores for each of the 23 chromosomes in every

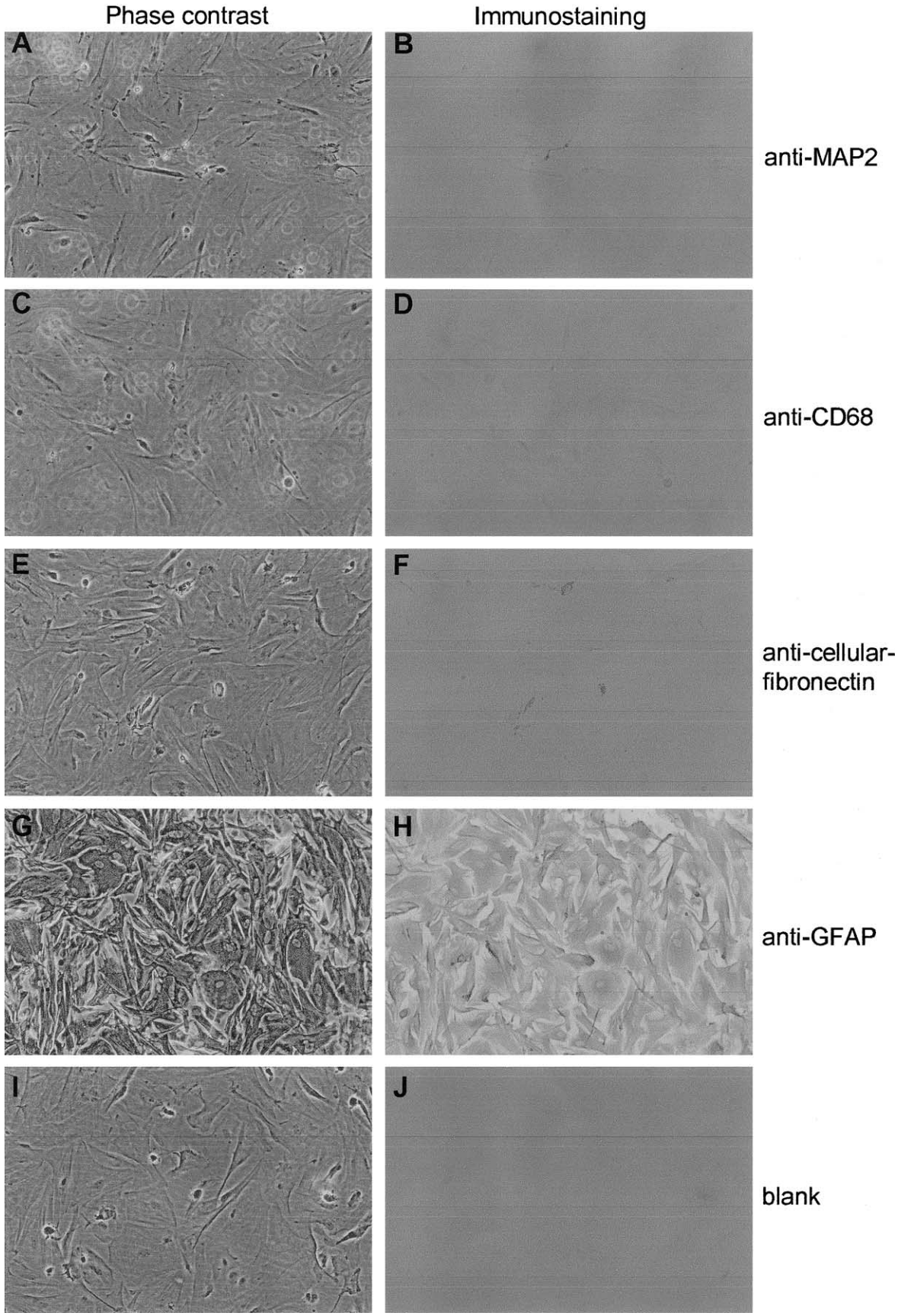
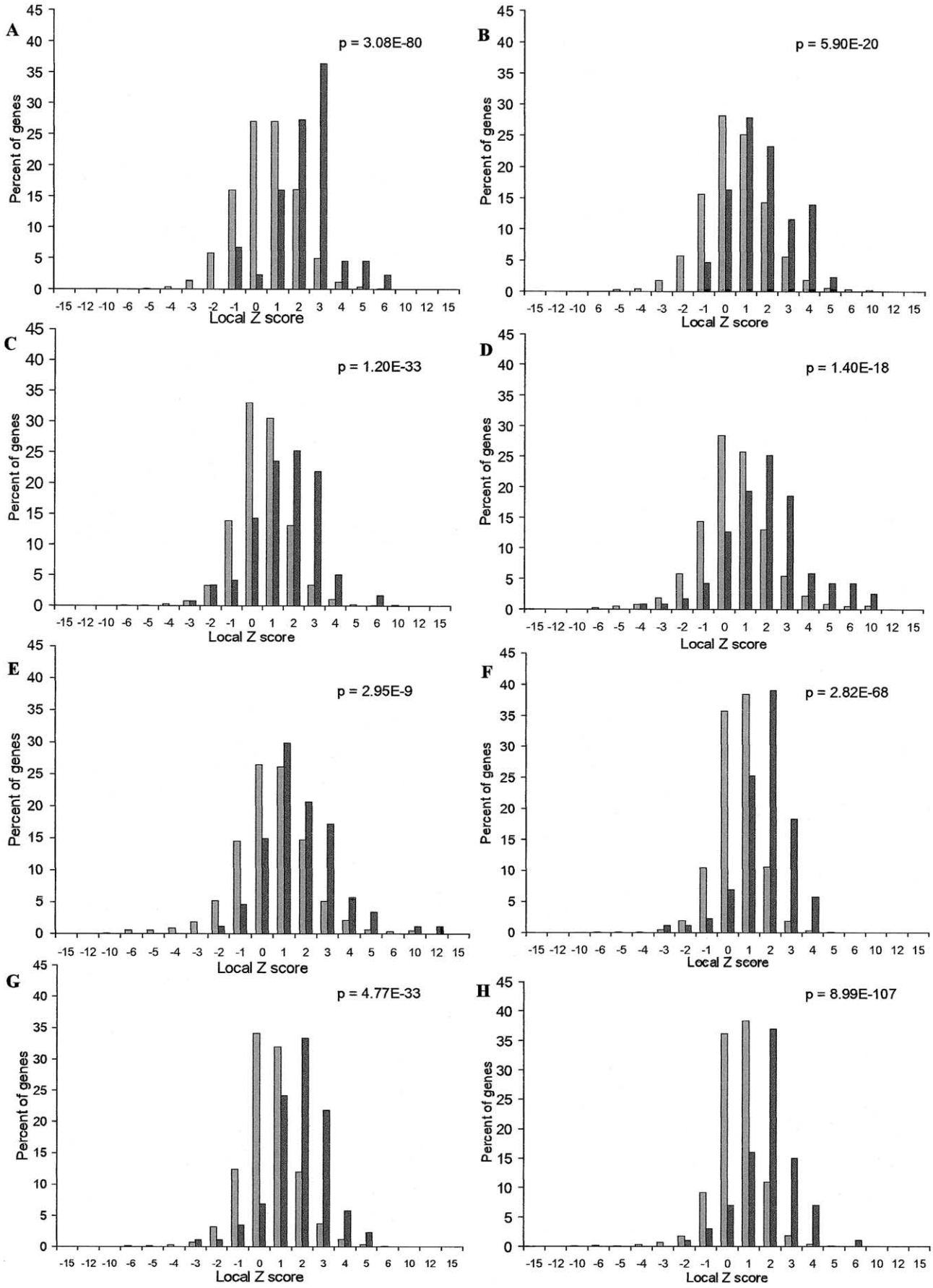


Fig. 1. Characterization of astrocyte cell lines by immunocytochemistry. Astrocyte cell line 848 (TS21) was stained with four antisera: (B) anti-MAP2 (a neuronal marker), (D) anti-CD68 (a macrophage marker; clone EBM11 monoclonal antibody), (F) anti-cellular fibronectin, (H) anti-GFAP (an astrocyte marker). (J) No primary antiserum was applied. (A, C, E, G, I) Corresponding phase-contrast images (original magnification ×50).


A
p = 3.08E-80
B
p = 5.90E-20
C
p = 1.20E-33
D
p = 1.40E-18
E
p = 2.95E-9
F
p = 2.82E-68
G
p = 4.77E-33
H
p = 8.99E-107
Percent of genes
Local Z score
-15 -12 -10 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 10 12 15

sample (see Materials and methods). For all eight samples, the genes assigned to chromosome 21 were robustly separated from genes assigned to all other chromosomes (Fig. 3A). In this PCA plot, the separation of chromosome 21 genes occurred along each of the first three principal components, which together accounted for 52.6% of the variance in the data. The plot is based on eight comparisons of TS21 to euploid controls. An example of a comparison of a single TS21 sample to matched euploid controls is shown in Fig. 3B. In this plot, the first principal component axis accounted for 29.7% of the variance in the data set and clearly separated the expression profile of genes on chromosome 21 from all other chromosomes (Fig. 3B). The plots in Figs. 3A and 3B also indicate that in TS21 cases, other chromosomes did not have this distinguishing gene expression profile. Similar analysis of euploid brain samples showed no abnormal gene expression profile from chromosome 21 or any other chromosome (Fig. 3C). In a comparison of euploid samples, the principal component axes accounted for a smaller percentage of the variance, further indicating that gene expression did not differ in euploid samples as a function of chromosomal localization.

The up-regulation of expression from chromosome 21 genes might have been either global or restricted to genes assigned to selective regions of chromosome 21. We generated a physical map of genes across the length of the chromosome 21q and observed a global pattern of up-regulation of gene expression for all eight TS21 samples (Fig. 4A). This was seen as an upward shift in values on the *y* axis (overall mean *Z* score was 1.32), consistent with the data in Fig. 2. The average local *Z* score for each gene is illustrated in Fig. 4B. Together, the analyses in Figs. 4A and 4B show that the overall up-regulation of gene expression we observed is global across chromosome 21 and is not restricted to particular chromosomal domains. In contrast to these findings, a comparison of gene expression in two euploid brain samples showed balanced levels of gene up- and down-regulation (Fig. 4C) with a mean *Z* score of −0.06. We obtained similar findings for other euploid–euploid comparisons (data not shown).

The variance in gene expression across chromosome 21 was greater in TS21 samples than in euploids. This is shown in Fig. 4D, in which the standard deviation of expression for each gene is plotted (mean values were 1.36 and 0.91 for TS21 and euploid samples, respectively). This increase in standard deviation was not observed for chromosomes other than 21 in TS21 cases, consistent with the results in Fig. 3A. However, the coefficient of variance calculated for the two groups (TS21 and euploid) was not significantly different (Fig. 4E). The coefficient of variance is a unitless, normalized description of the standard deviation, calculated as the ratio of the standard deviation and mean of the expression values. Almost all of the coefficient of variance values for TS21 samples are positive (Fig. 4E), because the mean local *Z* scores are positive (Fig. 4A). According to this measure, we conclude that while there is an increased mean in TS21 sample expression levels (Figs. 4A and 4B), the increase in standard deviation (Fig.4D) may be explained by the increased mean.

Notably, the data in Figs. 4A, 4B, and 4D suggest that few individual genes were consistently, selectively up-regulated. Instead gene expression was globally up-regulated. Analysis of gene expression values for all genes mapped to chromosome 21 indicates that some of the most consistently up-regulated genes in brain and astrocyte cell lines included Down syndrome critical region gene 2 (Hs.5198), superoxide dismutase 1 (Hs.75428), stress 70 protein chaperone (Hs.288799), ζ-crystallin (Hs.330208), cystatin B (Hs.695), and an ATP synthase (Hs.76572). A list of these genes is included in Supplementary Table 1.[1]

Several groups have generated transgenic mice that overexpress genes assigned to human chromosome 21. Those genes were selected for study in mouse models of DS because of their likely involvement in the DS phenotype. Thus, we asked whether the expression levels of these genes were elevated in the developing human brain. If expression levels were increased, this might provide independent support for the relevance of those mouse models to DS. Eight such transgenic mouse models are listed in Table 2 (adapted from [14]). We observed either modest elevated levels of

[1] Supplementary tables are available at http://pevsnerlab.kennedykrieger.org/index_ds.htm and doi:10.1016/S0888-7543(03)00035-1.

Fig. 2. Distribution of transcription levels (measured by local *Z* scores calculated from comparisons of a TS21 case to the appropriate euploid control) for genes on chromosome 21 compared to those of genes on other chromosomes in all TS21 samples. The percentage of genes (*y* axis) is plotted versus the *Z* score (*x* axis) calculated with SNOMAD software. Each plot shows the profile of expression of genes assigned to chromosome 21 (dark bars) and all other genes (light bars) for the comparison of one TS21 sample to the appropriate matched control(s). The *p* values were calculated from a χ^2 test. For each plot, gene expression values are from TS21 samples and euploid controls as follows: (A) TS21 astrocyte (sample 907) compared to euploid astrocyte (sample 700). (B) TS21 astrocyte (sample 848) compared to euploid astrocyte (sample 696). (C) TS21 astrocyte (sample 1478) compared to the average of two euploid astrocytes (samples 1521 and 1479). (D) TS21 astrocyte (sample 748) compared to the average of two astrocytes (samples 1521 and 1479). (E) TS21 brain (sample 847) compared to the average of four euploid brain tissue samples (see Table 1). (F) TS21 brain (sample 1218) compared to the average of four euploid brain tissue samples. (G) TS21 brain (sample 1389) compared to the average of four euploid brain tissue samples. (H) Genes from TS21 brain (sample 1478) compared to the average of four euploid brain tissue samples. For (A) and (B) Affymetrix U95Av2 chips were employed and the number of genes annotated on chromosome 21 with detectable expression levels was 44 (A) or 43 (B) and the total number of other genes was 3923 (A) or 4201 (B). For (C–H) Affymetrix U133A chips were employed and the number of genes annotated on chromosome 21 with detectable expression levels was 119 (C, D) or 87 (E–H) and the total number of other genes was 10,781 (C, D) or 8230 (E–H). Note that one TS21 astrocyte cell line (C) and one brain sample (H) are from the same individual.

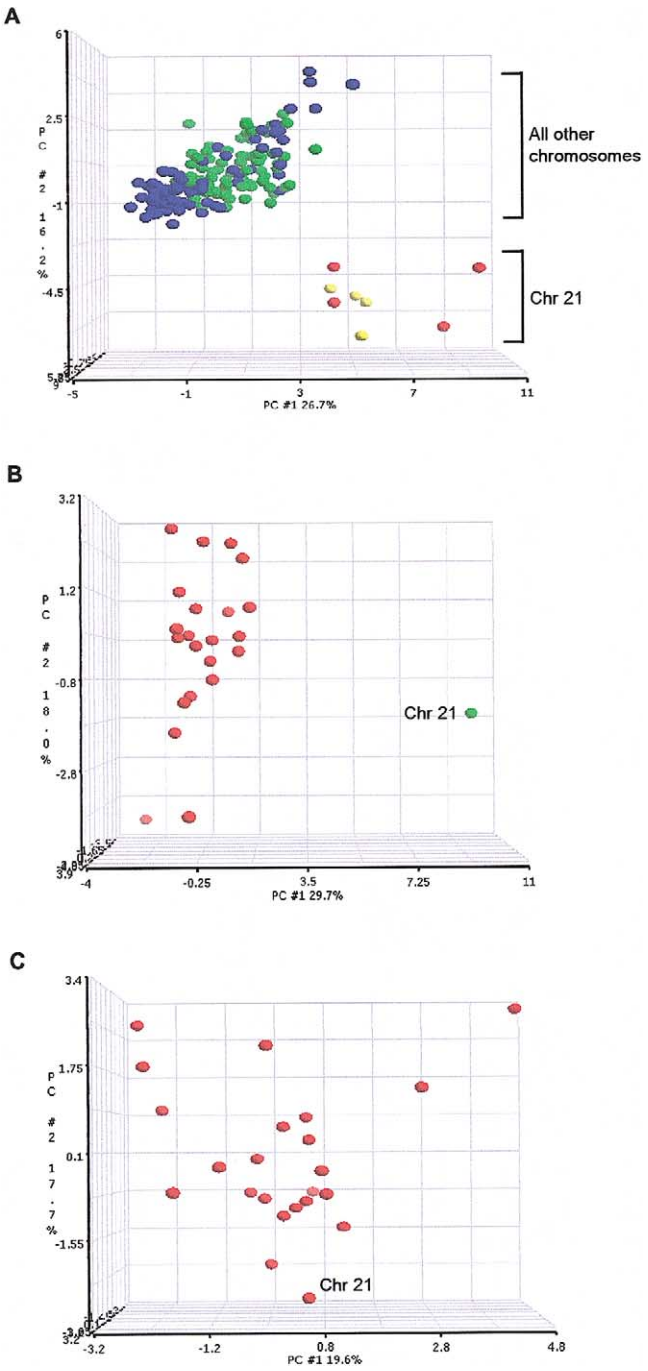


Fig. 3. PCA distinguishes the expression pattern of genes localized to chromosome 21 from all other chromosomes in TS21 samples. PCA was based on local *Z* scores derived from SNOMAD analysis (see Materials and methods). Each circle represents a chromosome (1–22, X); only chromosome 21 is labeled. (A) PCA representation of gene expression in four TS21 brain samples relative to euploid controls (genes assigned to chromosome 21 are colored yellow; genes assigned to all other chromosomes are colored blue) or four TS21 astrocyte cell lines relative to euploid controls (genes assigned to chromosome 21 are colored red; genes assigned to all other chromosomes are colored green). The first three principal components accounted for 52.6% of the data variance. (B) As an example of PCA visualization of an individual comparison, the plot reveals the differential regulation of genes assigned to chromosome 21 relative to all other chromosomes from a comparison of a TS21 brain (sample 847) to the average expression values of four euploid brain samples. The first three principal components accounted for 58.9% of the data variance. (C) PCA representation of gene expression in a euploid brain (sample 1521) compared to another euploid control (sample 1565). The first three principal components accounted for 50.3% of data variance. The position of chromosome 21 was not significantly separated from other chromosomes nor was gene expression on chromosome 16 (corresponding to the data point at top right of C) significantly regulated.

expression in DS samples relative to euploid controls or low levels of expression below detection limits (Table 2).

In another approach to identifying genes that are regulated in DS, Bahn et al. performed differential display analysis on neuronal precursor cells derived from the cortices of three fetuses with DS [15]. They identified several genes that were abnormally regulated. None of these genes was significantly regulated in our studies of brain tissue samples or cell lines (Supplementary Table 2).

Brain is composed of multiple cell types, including astrocytes. We asked whether the gene expression changes we observed in chromosome 21 were consistent among brain and astrocyte samples. For the 12 samples analyzed with the same U133A microarray chip, PCA analysis revealed four groups (Fig. 5). Across the first principal component axis (*x* axis), all astrocyte samples were distinguishable from brain samples. Across the second principal component axis (*y* axis), all TS21 samples were distinguishable from all euploid samples. This suggests that TS21 samples can be distinguished from euploid samples based upon the variance of gene expression values. The systematic shift across the second principal component axis in both brain tissue and astrocyte cell lines suggests that the gene expression differences between TS21 and euploid states are comparable in the two tissue sources.

Discussion

We observed a global up-regulation of gene expression from chromosome 21 in TS21 brain-derived samples. This finding supports the hypothesis that a global chromosome 21 dosage imbalance causes the heterogeneous phenotypes of DS [5,6]. It remains possible that the overexpression of a limited number of genes on chromosome 21 is responsible for the DS phenotypic features [7]. The dramatic, global up-regulation of chromosome 21 genes in TS21 cases we observed was specific to that chromosome.

While we observed consistent, statistically significant global overexpression of genes on chromosome 21, the magnitude of the change of individual genes was modest (Supplementary Table 1). This is consistent with a model in which an extra copy of chromosome 21 causes a 1.5-fold increase in transcription by means of a dosage effect. For several genes described as down-regulated in human neuronal precursor cells by Bahn et al. [15], either we observed minor changes in expression levels of those genes or our microarray assay did not detect expression of those genes

above background (Supplementary Table 2). The differences between our results and those of Bahn et al. could be due to the use of entirely different experimental paradigms, different cell types, and different gestational ages.

There are several potential limitations to the approaches we have taken. There is a limit to the sensitivity of microarrays. Thus, it is conceivable that some genes having important roles in DS are not expressed at detectable levels. Not all annotated genes are represented on the microarrays. Furthermore, our studies focused on brain samples and astrocyte cell lines from 17 to 21 gestational weeks. While this is an important age, it is possible that other changes occur at different developmental time periods. Our data do not address the question of changes in protein levels [16], but only in mRNA levels. It is possible that the mRNA levels do not have a strong positive correlation with the corresponding protein expression levels [17–19]. Proteomic analysis of the TS21 developing brain should complement the genomic data reported here. Finally, the control cases were African American, whereas the TS21 were Caucasian. We selected cases based on availability from the Brain and Tissue Bank for Developmental Disorders. We are aware of no evidence suggesting any gene expression changes that are based on racial differences.

Our observations were made in both cultured astrocytes derived from postmortem brains and brain homogenates. These distinct biological sources revealed similar transcriptional responses in DS brain. The differences in gene expression between brain samples and astrocytes (Fig. 5) could be due to the multiple cell types present in the brain. These differences could also be caused by changes associated either with the growth of cultured cell lines or with frozen brain samples [20].

DS is characterized by both an extraordinarily broad set of phenotypic features and a highly variable expression of these features among affected individuals [2]. For example, increased variability in the cranial morphology has been reported in DS adult males [21]. Similarly, Ts65Dn, a mouse genetic model of DS, exhibited a higher degree of mandible variance than euploids [22]. Here we have shown from the RNA level that there is also greater variability in gene expression levels on chromosome 21 among DS fetuses than among euploid controls. This increase in variability is likely due to an increase in gene expression levels. We speculate that the increased standard deviation in expression levels of DS genes across chromosome 21 (Fig. 4D), together with the global up-regulation, reflects a process in which variable levels of transcription contribute to the increased phenotypic variability of DS. We note that the coefficient of variance calculation (Fig. 4E) normalizes the standard deviation measure as a function of gene expression level and does not indicate a greater variance in gene expression in TS21 samples relative to euploid controls. For genes that are more highly expressed, there may be a greater inherent variance (Fig. 4E). Thus, we suggest that the consistent increases in standard deviation across chromosome 21 (Fig. 4D) depend in part on the expression levels of the genes.

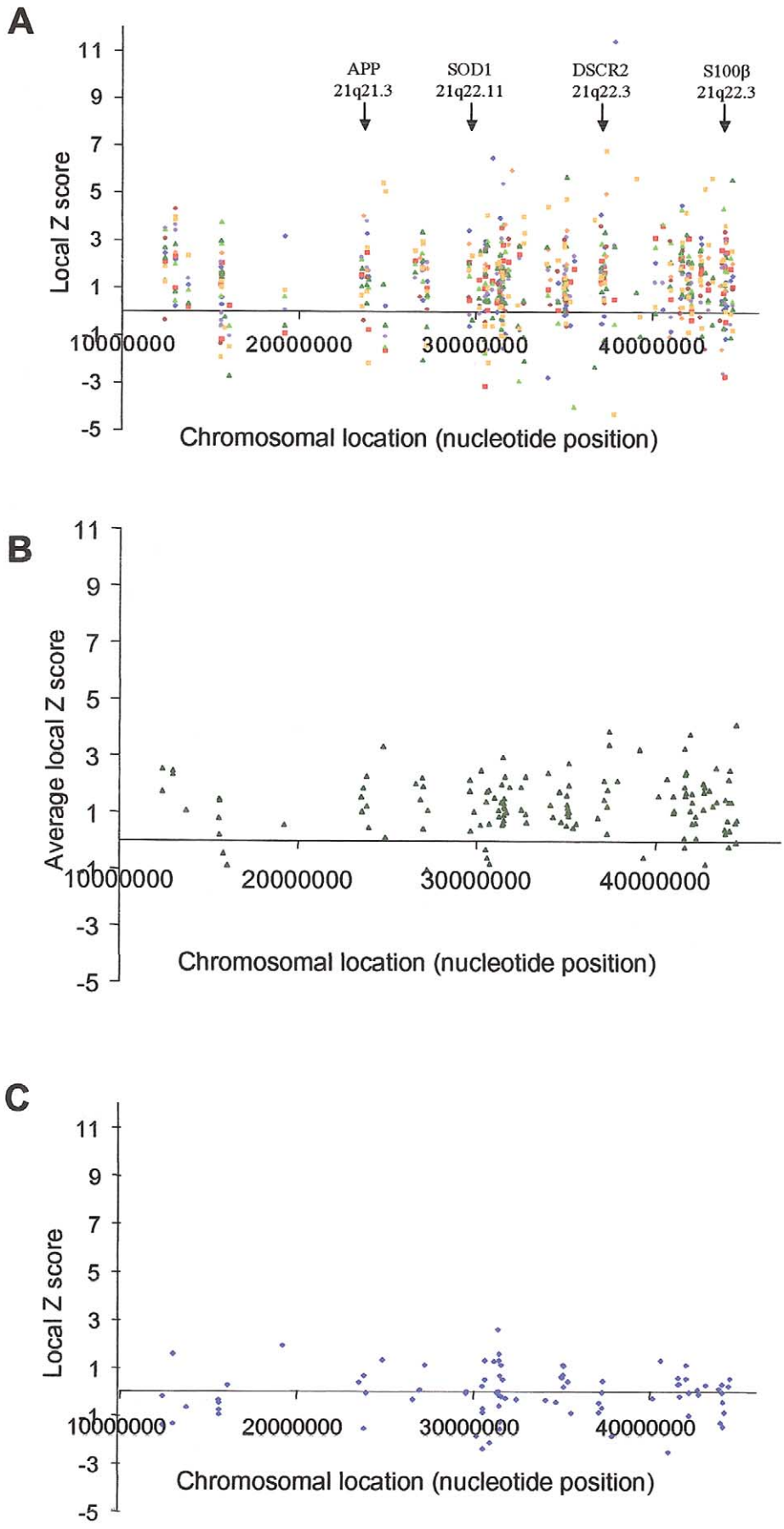
We have described the analysis of gene expression as a function of chromosomal locus. This approach could be applicable to studies of other trisomies (e.g., trisomy 13 and 18) or other aneuploidies. We speculate that patterns of global up-regulation similar to those we have observed might also exist in these trisomies. In addition, our study was performed on samples derived from one organ (brain) at a particular time during development (17 to 20 gestational weeks). It would also be relevant to examine gene expression profiles of patient-derived samples from other tissues or cell types and from other ages, which could provide insight into regional specificity and age-related changes in expression in DS.

Materials and methods

Samples. All human tissue was obtained from the Brain and Tissue Bank for Developmental Disorders at the University of Maryland with informed consent using Institutional Review Board-approved protocols. Diagnoses, gender, race, and other information are provided in Table 1. For four of the DS samples and four age-matched controls, cerebral cortex was dissected and astrocytes were isolated and cultured in Dulbecco's modified Eagle's medium [23,24]. After two passages, the purity of the cultures was confirmed at the time of cell harvest by immunocytochemistry as described [10]. Four additional DS samples and four age-matched controls were frozen and the cerebrum or apical frontal pole was dissected. RNA was extracted from cell lines (at 80–90% confluency) or from brain (150 mg wet weight) using the RNeasy Midi Kit (Qiagen). The quantity and purity of RNA were confirmed by spectrophotometry and agarose gel electrophoresis.

Gene expression data acquisition and analysis. Gene expression data were obtained using Affymetrix U95Av2 or U133A chips with standard protocols at the Johns Hopkins Microarray Core Facility. All raw expression data are provided in Supplementary Tables 3 and 4, and descriptions of the most highly regulated genes are presented in Supplementary Tables 5 and 6. In Fig. 2, gene expression was measured for each TS21 astrocyte cell line relative to matched euploid cell lines that were obtained, grown, and processed at the same time. Thus the matched controls differ in Figs. 2A–2D. Local Z scores, in standard deviation units, were calculated with local background corrections using SNOMAD [25] (http://pevsnerlab.kennedykrieger.org/snomad.htm). A Z score of $+2$ represents a gene expression ratio between two samples (e.g., TS21 and euploid) that is 2 standard deviations above the mean. The p values were calculated from χ^2 tests.

PCA and t tests were performed using Partek Pro software (http://www.partek.com). For Fig. 3, the percentages


A
APP
21q21.3
SOD1
21q22.11
DSCR2
21q22.3
S100β
21q22.3
Local Z score
11
9
7
5
3
1
-1
-3
-5
10000000
20000000
30000000
40000000
Chromosomal location (nucleotide position)
B
Average local Z score
11
9
7
5
3
1
-1
-3
-5
10000000
20000000
30000000
40000000
Chromosomal location (nucleotide position)
C
Local Z score
11
9
7
5
3
1
-1
-3
-5
10000000
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Chromosomal location (nucleotide position)

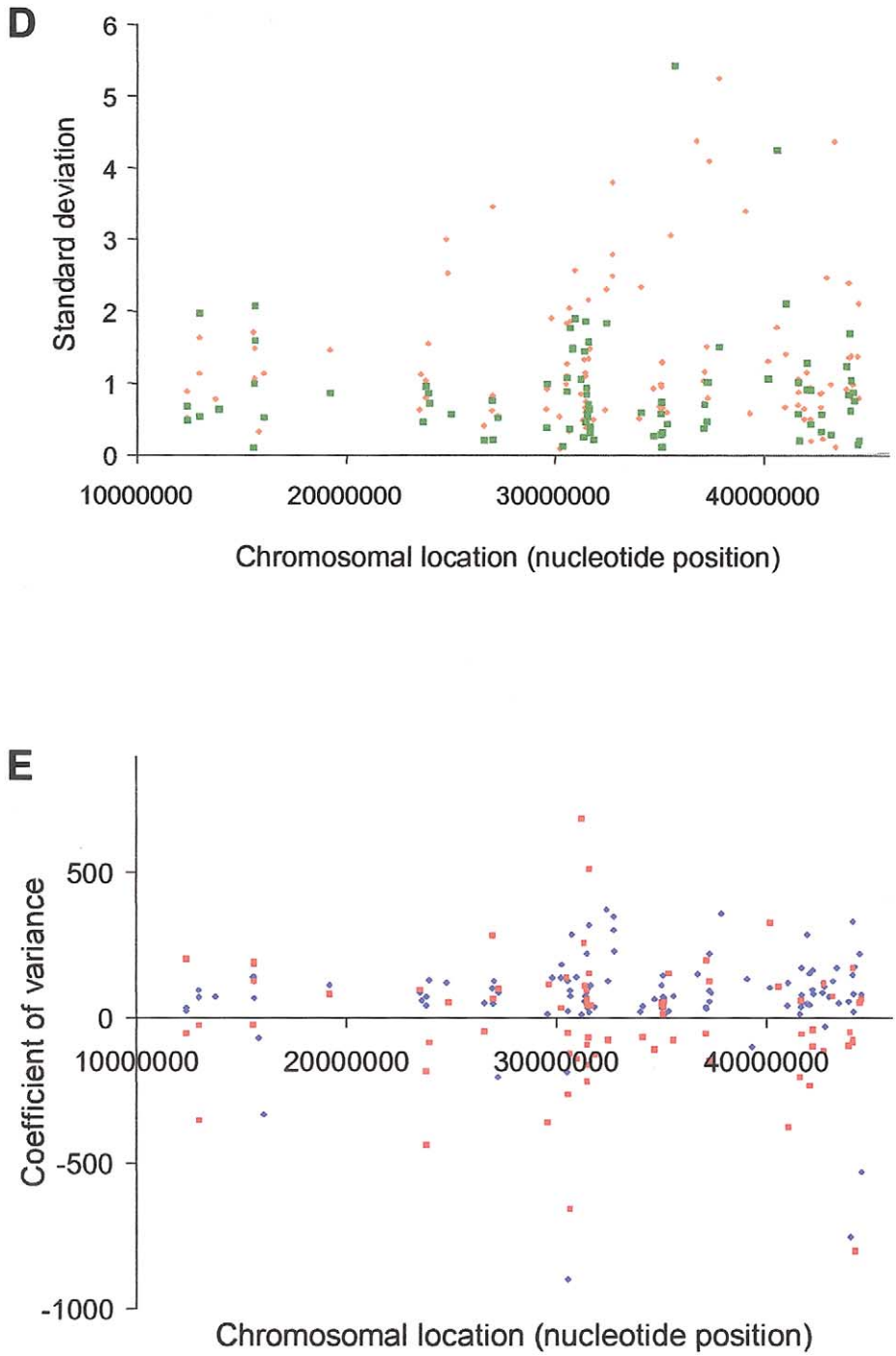

Fig. 4 (*continued*)

Fig. 4. Expression levels of genes across chromosome 21 were globally shifted upward in TS21 samples compared to controls. The genes on chromosome 21 for which expression values were obtained are represented on a physical map (*x* axis; telomere to the right) as a function of *Z* score (*y* axis). Units are nucleotide position from the NCBI Map Viewer (http://www.ncbi.nlm.nih.gov/). (A) Expression levels of genes across chromosome 21 in eight TS21 samples. Symbols in different colors represent four TS21 cultured astrocyte cell lines and four TS21 brain tissues. Key: blue diamond, brain 847 versus euploids; red square, brain 1218 versus euploids; light green triangle, brain 1389 versus euploids; purple circle, brain 1478 versus euploids; orange diamond, astrocytes 907 versus 700; red circle, astrocytes 848 versus 696; dark green triangle, 1478 versus euploids; orange square, astrocyte 748 versus euploids. (B) Average of the local *Z* scores for each gene (from A) across chromosome 21. There is one data point for each gene. (C) Expression levels of chromosome 21 genes in a comparison of two representative euploid brain samples (1521 and 1565). Note that the mean local *Z* score is −0.06. (D) Standard deviation of local *Z* scores for eight TS21 samples compared to euploid controls (*y* axis) across chromosome 21 (*x* axis). The plot shows a greater range of standard deviations in TS21 samples (orange diamonds) than in euploid samples (green squares). (E) The coefficient of variance was calculated for the data in D and plotted as a function of chromosomal position for TS21 samples (blue diamonds) and euploid samples (red squares).

Table 2
Expression levels of individual chromosome 21 genes in human brain and astrocytes that have previously been assessed in transgenic mouse models.

Gene name	Mouse model	Phenotype in mouse model	Expression in human brain[a]	Expression in human astrocytes[b]
Superoxide dismutase 1	TgSod1 [26]	Learning defects	1.75 ± 1.27	1.64 ± 1.28
Phosphofructokinase, liver	TgPfk1 [27]	—	1.23 ± 1.19	1.07 ± 1.08
S100β	TgS100β [28]	Astrocytosis, neurite degeneration	Below background level	1.26 ± 1.48
Amyloid precursor protein	TgApp [29]	Cognitive/behavioral defects	1.66 ± 1.23	1.57 ± 1.18
Ets2	TgEts2 [30]	—	Below background level	1.71 ± 4.77
HMG-14	TgHmg14 [31]	—	1.29 ± 1.36	1.44 ± 1.15
Minibrain	TgMnb [32]	Learning/memory defects	1.46 ± 2.55	1.33 ± 1.86
Single-minded 2	TgSim2 [33]	Behavioral defects	Below background level	Below background level

The list of mouse models and corresponding phenotypes is adapted from [14].
[a] Average signal ratio (TS21:euploid) from U133A chips.
[b] Average signal ratio (TS21:euploid) from U95Av2 chips.

of genes with various Z scores (defined using 18 bins ranging from $Z < -15$ to $Z > +15$) were compiled into a spreadsheet in Microsoft Excel for each individual chromosome in each sample. In Fig. 3A there were 23 chromosomes × 8 samples = 184 data points. In Fig. 3B there are 23 data points, shown from one comparison of TS21 (sample 847) to euploid (average of four controls). PCA was performed by importing each Excel spreadsheet into Partek Pro using default settings (correlation dispersion matrix, normalized eigenvector scaling, R-analysis mode).

For Fig. 4, we created a physical map by downloading the NCBI Map Viewer on a Dell workstation (Red Hat Linux operating system) and searching each gene accession number against the chromosomal locus. These maps were created in Microsoft Excel. For Figs. 4D and 4E, we calculated the standard deviation and coefficient of variation in Excel. The standard deviation was calculated from all eight TS21 samples compared to matched euploid controls and separately from pairwise comparisons of euploid samples to other matched euploid samples for which microarray data were collected at the same time. The coefficient of variance was calculated as (standard deviation/mean) × 100.

We identified genes assigned to chromosome 21 that are significantly up-regulated (see Results and Supplementary Table 1) using two criteria. First, we used Affymetrix software to select genes that were called present. Second, we selected genes having an absolute value ≥2 for all local Z-score measurements.

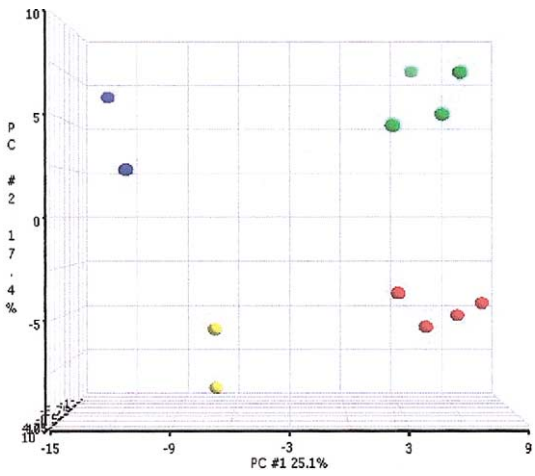

Fig. 5. PCA of chromosome 21 genes showing grouping by sample types. PCA was based on normalized signal intensities from Affymetrix microarray experiments (Microarray Suite version 5.0). Results of four cultured astrocyte cell lines (blue, TS21; yellow, euploid) and eight brain tissues (green, TS21; red, euploid) analyzed with human U133A gene chips. The three principal components accounted for 53.5% of the total variance in the data. A similar separation using TS21 and euploid samples was observed for gene expression data from six cultured astrocyte cell lines using Affymetrix U95Av2 chips (not shown).

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