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Gene Expression Profile Analysis in Human T Lymphocytes from Patients with Down Syndrome

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Summary

Down Syndrome (DS) is caused by the presence of three copies of the whole human chromosome 21 (HC21) or of a HC21 restricted region; the phenotype is likely to have originated from the altered expression of genes in the HC21. We apply the cDNA microarray method to the study of gene expression in human T lymphocytes with trisomy 21 in comparison to normal cells.

Two patients with DS were investigated, along with two normal subjects as a control, all being tested in independent, duplicated cell culture experiments. The most consistent finding was the overexpression of the superoxide dismutase gene (*SOD1*), located on 21q, and of MHC DR beta 3 (*HLA-DRB3*), GABA receptor A gamma 2 (*GABRG2*), acetyltransferase Coenzyme, A 2 (*ACAT2*) and ras suppressor protein 1 (*RSU1*) genes. When the data were clustered according to chromosome localization, the HC21 gene set showed, on average, the highest expression in DS cells in all the experiments. Moreover, separate clustering of patients and controls was obtained when analysis was restricted to HC21 gene expression values.

These findings reinforce the specific gene dosage theory for the pathogenesis of the DS phenotype, and show a consistent overexpression of the *SOD1* gene on 21q.

Introduction

Down Syndrome (DS) is the most frequent form of mental deficit due to a chromosomal aberration; recent data estimates an incidence of one DS individual per 400 conceptuses (Merrick, 2000). The disease is caused by the presence of three copies of the whole human chromosome 21 (HC21), or of a HC21 restricted region, and is associated with heart and gastrointestinal malformations, increased risk of leukemia, and deficits of the immune and endocrine systems. The patients show a typical facies and early onset of Alzheimer disease (McKusick, 1998; Korenberg *et al.* 1994).

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The phenotype probably originates from altered expression of the genes in HC21. The Down Syndrome Candidate Region (DSCR) includes 10% of HC21, spanning the 21q22 region (Korenberg, 1994; Delabar *et al.* 1993; Antonarakis, 1998); its triplication has been shown to be sufficient for the DS phenotype in some subjects. Very little is known about the molecular pathogenesis of Down Syndrome in terms of the contributions of single genes to the DS phenotype. To date, only two studies have been performed using cDNA microarrays in human cells with trisomy 21, one in fetal cells (FitzPatrick *et al.* 2002) and one in placentas of pregnancies with trisomy 21 fetuses (Gross *et al.* 2002).

The aim of this work was the application of the cDNA microarray method to study the gene expression profile in human blood lymphocytes with trisomy 21 compared to normal cells, to search for single gene expression or

expression profiles specific for aneuploidy. We present the first characterization of a gene expression profile in human T cells with trisomy 21, with implications for the gene expression profile in aneuploid differentiated cells, and for the possible specific alterations of T lymphocyte immune cells in patients with DS.

Materials and Methods

Cells

The cellular samples were kindly provided by the 'Galliera Genetic Bank', funded by the Telethon Foundation at the Laboratory of Human Genetics, Galliera Hospital, Genova (thanks to Dr. Chiara Baldo). The samples were primary cell cultures of human T lymphocytes, obtained from 10 mL peripheral blood samples. The low-density mononuclear cells were isolated by density gradient separation (1.077 g/mL), then seeded at 1×10^6/mL concentration in RPMI medium with 10% AB human serum and 0.12 μg/mL phytohemoagglutinin (PHA). After three days, the cells were washed and seeded at 0.5×10^6/mL concentration in RPMI medium with 10% AB human serum and 10 ng/mL recombinant human interleukin 2 (rhIL-2, Roche Diagnostics, Basilea, Switzerland). The cells were expanded in these conditions, then frozen. For the experiments, the cells were unfrozen, cultured with IL-2, and shipped at room temperature. After delivery, they were incubated overnight at 37°C, and then total RNA was extracted.

Cell cultures and respective recorded data available at the Bank were: two cultures from DS patients (NA99110F, female, less than 10 years old, abbreviation: DS1; D99106M, male, 12 months old, abbreviation: DS2); two cultures from normal subjects: AA00160F, (female, less than 35 years old, abbreviation: N1; AG00161M, male, 32-40 years old, abbreviation: N2).

Two independent microarray experiments were conducted, starting from two different thawing and culture sessions; in experiments #1 and #4 we hybridized DS1 and N1 RNAs from first and second cell thawing and culture, respectively; in experiments #2 and #3 we hybridized DS2 and N2 RNAs from first and second cell thawing and culture, respectively.

Cytofluorimetric analysis

To evaluate the purity of the cell cultures, 1 million cells for each cell culture were subjected to cytofluorimetric analysis, courtesy of Dr. Damiano Rondelli (Institute of Hematology, University of Bologna). Cells were labelled with monoclonal antibodies anti-CD3, − CD56, − CD8 and − CD4, specific for T-lymphocytes (CD3+, CD3+/CD56+, CD8+, CD4+) and NK cells (CD3-/CD56+). The analysis was conducted using the flow cytometer FACSCalibur (Becton Dickinson, San Jose, CA), with the software Cell QuestTM.

RNA

About 40 million lymphocyte cells were collected in a laboratory centrifuge at 2,000 rpm for 5 min. The pellet was subjected to total RNA extraction, according to the method of Chomczynski & Sacchi, (1987), adding 5 mL of D solution to the polypropilene tube with the pellet. The final RNA pellet was dissolved in 14-16 μL bidistilled sterile water. To evaluate the RNA quantity and integrity, 1 μL of the suspension was electrophoresed onto a 2% agarose gel with 0.5 μg/mL ethidium bromide.

'MicroMax' human array hybridization

For the analysis using the commercial glass microarray 'MicroMax' NEN, each RNA sample was labelled with Cy3 (green, samples from DS patients) or Cy5 (red, normal samples). Briefly, 50-75 μg of total RNA from each sample were reverse transcribed and labelled with the reagents provided with the slides.

Following the labelling reaction, the two cDNAs labelled with Cy3 and Cy5, respectively, were mixed and the final volume ($\sim$80 μL) was applied to a Quick-spin G-25 Sephadex purification column (Roche Diagnostics), following the manufacturer's instructions. The eluted cDNAs were precipitated in sodium acetate 0.3 M and 1 volume of cold ethanol, and then directly resuspended in 20 μL of hybridization buffer (see below). This mix was denatured at 90°C for 2 min and briefly centrifuged, then loaded into the hybridization chamber (purchased from TeleChem, Sunnyvale, CA).

We used a hybridization aqueous solution with a high Sodium Dodecyl Sulfate (SDS) concentration, according to Church & Gilbert (1984), except that albumin was omitted. The hybridization was performed at 65°C with the chamber submerged in a thermostatic bath. The slide was washed at room temperature by gentle agitation in a plastic tray with high-stringency wash solution (5% SDS, 20 mM Sodium phosphate, and 1 mM EDTA pH 8) and warmed at 65°C twice, for 5 min. The array was then placed in a 50-mL tube and dried first by centrifugation in a clinical centrifuge (1000 rpm for 30-60 sec), and then for 1 hour at 52°C. In preliminary test experiments, the Church & Gilbert protocol gave us better results than those obtained when the buffers included in the NEN kit were used.

Array image and data analysis

The array was scanned with a GenePix 4000A (Axon, Union City, CA) reader. If necessary, further washes were performed to reduce the aspecific background. The image was analyzed using the software GenePix 3.0, with visual inspection to exclude spots in the drying lanes. The median of the pixel intensities for each spot and for the corresponding surrounding area (background) was obtained for each channel.

Three main types of analysis were performed. First, we imported data into the FileMaker Pro 5 database (FileMaker Inc., Santa Clara, CA) to search for over- or underexpression of specific genes that was consistent for each patient and for each duplicate experiment. Moreover, using the FileMaker Pro version of Unigene, we defined a relationship in the database to assign a location (undeclared by the manufacturer) to 2185 out of 2400 present in the array, to analyze data clustered for map position. Finally, data relative to the red (controls) and green (patients) channels were subjected to cluster analysis to define relationships between the expression profiles using the software EPCLUST (http://ep.ebi.ac.uk/EP/EPCLUST/).

Statistical analyis was performed by SPSS (SPSS, Chicago, IL) software, using the t-test and rank sum test (gene-stratified) on the 334-gene normalized final dataset (see below).

Results

Cytofluorimetric Analysis

The cytofluorimetric analysis of the cellular samples confirm the high purity of T cells in all samples; the data for each cell culture were (see Methods for the abbreviations): DS1: CD3+ = 99%, CD3+/CD56+ = 8%, CD8+ = 55%, CD4+ = 45%, CD3-/CD56+ = 0; DS2: CD3+ = 85%, CD3+/CD56+ = 32%, CD8+ = 66%, CD4+ = 33%, CD3-/CD56+ = 15%; N1: CD3+ = 97%, CD3+/CD56+ = 13%, CD8+ = 78%, CD4+ = 21%, CD3-/CD56+ = 2%; N2: CD3+ = 98%, CD3+/CD56+ = 32%, CD8+ = 87%, CD4+ = 12%, CD3-/CD56+ = 2%.

'MicroMax' Array Hybridization and Data Analysis

The hybridization protocol (Church & Gilbert, 1984) based on high temperature and high stringency washes allowed us to obtain hybridizations with a low level of background (Fig. 1). The dataset used as the base for the analysis was the 'results' file provided by the software GenePix 3. The 'background median' was the median of the pixel intensities in the area surrounding the spot, while the 'feature median' is the median of the pixel intensities in the area inside the spot. The spot intensity was then calculated by subtracting the background median value from the feature median value. The original datasets for experiments 1 and 4 (independent replication of the DS #1 patient versus normal (N) #1 subject experiment, see Methods) and experiments 2 and 3 (independent replication of the DS #2 patient versus N #2 normal subject experiment) are provided as supplementary data at the address: http://apollo11.isto.unibo.it/suppl/giannone2004/.

After data importation into a relational database management system (FileMaker Pro 5 for the Macintosh), we checked and normalized the data. For the analysis, we considered only the genes (out of 2,400 total probes) matching the following criteria: availability of the full series of 8 measurements (four slides per two channels), all with positive (greater than 0) values for the "spot intensity" calculated by GenePix software as above; absence in all experiments of any related spot

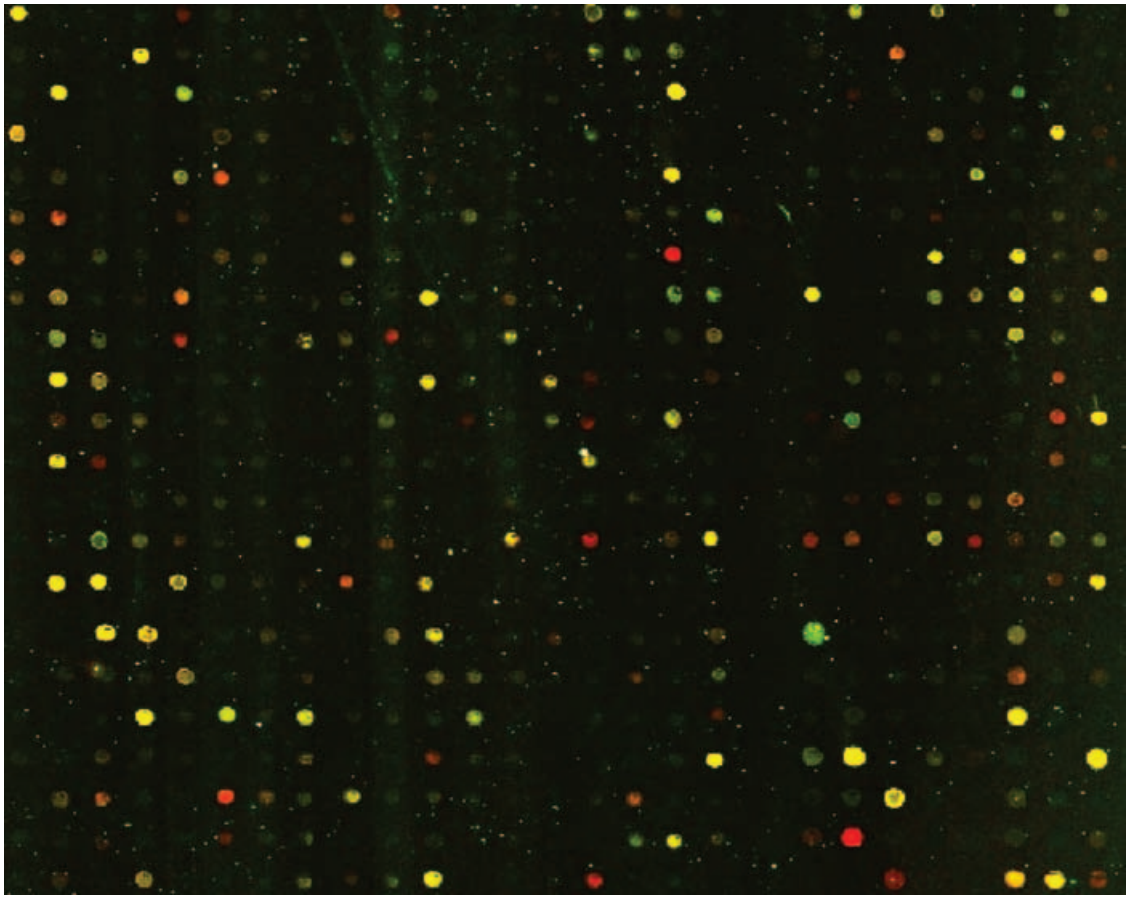

Figure 1 Array hybridization: appearance of a MicroMax microarray region (experiment no. 1) after washes and laser scanning.

visually localized in areas with artifacts (Fig. 2); absence in all experiments of any related spot called as "not found" by the GenePix software, by internal criteria including number of pixels above background threshold or feature diameter - usually this means that expression level cannot be affordably assessed, for biological (no expression) or technical (artifactual area) reasons.

The resulting 334-gene dataset was then normalized on the basis of the expression of housekeeping genes. For each slide, we determined the red/green ratio for 34 genes: *GAPD*, beta actin and 32 ribosomal proteins, available on MicroMax microarray. The average of the red/green fluorescence ratio for these genes was used as a correction/normalization factor for each slide. The validity of the correction was tested verifying the resulting ratio for other known housekeeping genes, such as histone H3 or TATA binding protein genes; the value was 1 or close to 1. The final 334-genes normalized dataset is available as a supplementary material.

To identify genes significantly over- or underexpressed in trisomic cells, we considered the mean expression ratio for each gene in the four experiments (two biological replicates for each patient/normal pair). A differential expression between cases and controls was considered biologically significant when consistent in both patients and in both experiments for each patient. As a threshold value, for significance we used the arithmetic average of the red/green intensity plus/minus 1 standard deviation. Results are described in Table 1, listing the genes relatively overerexpressed in DS cells (no gene was underexpressed by these criteria). Statistical analyis for significance is also reported, fully confirming the overexpression of genes observed with the arbitrarily chosen threshold (Table 1). We present evidence that for several genes, the arbitrary "descriptive"

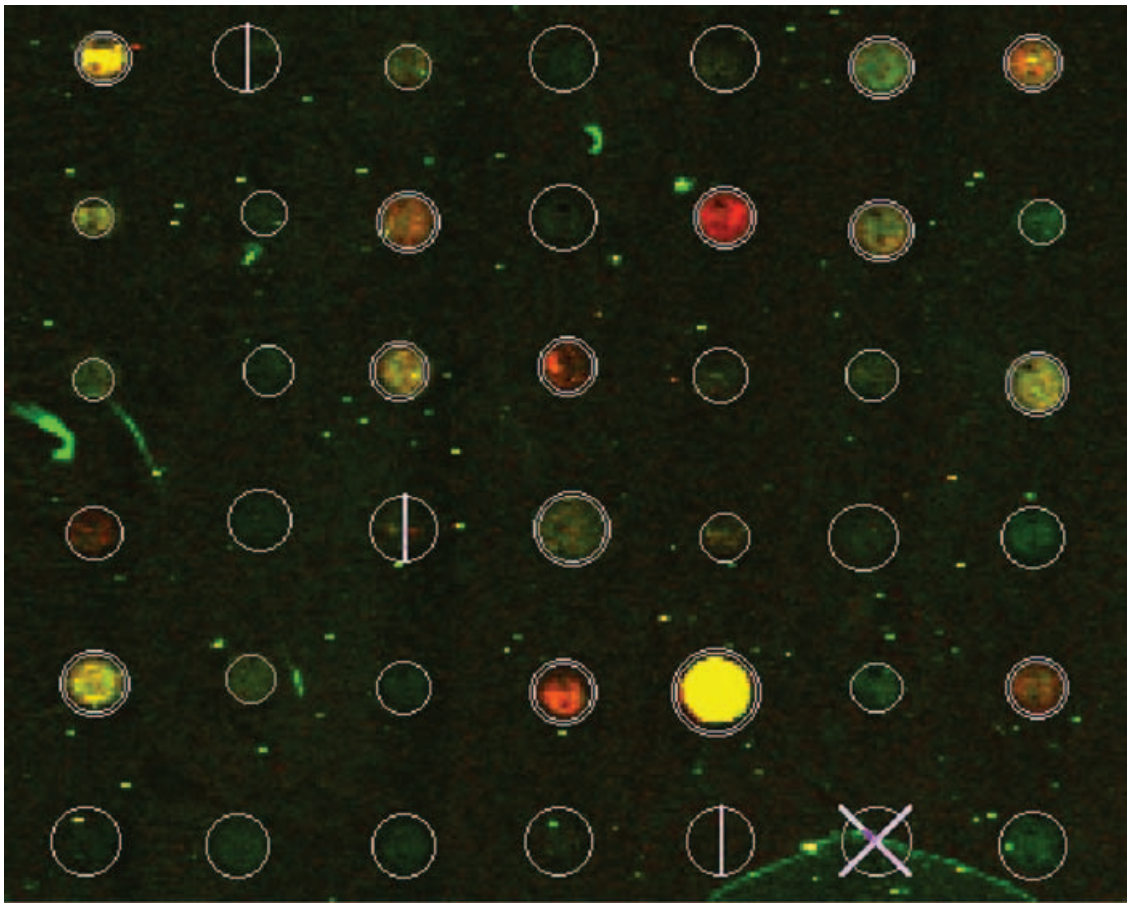

Figure 2 Array spot revision: manual revision of spot quality using GenePix 3 software. Circle: 'clear' spot (Flag '0'; double circle: 'good' spot (manually assigned, Flag '100'); vertical bar: spot not found (absence of detectable hybridization); cross: low quality due to artifacts.

cutoff of mean ± 1 standard deviation actually possessed statistical significance in terms of $p<0.05$. Gaussian profile (Kolmogorov-Smirnov test) was obtained using the 334-gene set on which the statistical analysis was performed. In addition, due to the fact that the low number of samples may question the assumption of a parametric distribution of the variable "spot intensity," we also performed a nonparametric test (rank analysis) for significance of overexpression, with similar results. Interestingly, the mean rank observed in DS subjects for all genes with a significant p-value (Table 1) was $(5+6+7+8)/4 = 6.5$ versus $(1+2+3+4)/4 = 2.5$ observed in normal subjects, further underlining the differences between cases and controls.

We also studied differential gene expression according to chromosomal location. For this purpose, we compared the expression values for 334 spots corresponding to genes of known location, whose abundance was adequate to allow an assessment of the expression level. The spots were selected for this analysis only if their quality was rated as at least 'clear' (GenePix tag '0', assigned by the software) in all experiments, and if intensity values were positive in all experiments for both channels. Mean expression ratios between normal and trisomic cells, related to chromosomal location, are listed in Table 2. While the global ratio observed in the whole genomic profile is very close to 1, the chromosome whose genes show on average the highest expression in the DS patients in comparison to normal subjects, considering all the experiments, is HC21 (average of normal/DS ratios 0.697 ± 0.274 standard deviation; Table 2); however, this finding was not statistically significant.

Independently of their inclusion in the previous analysis, the normal/DS expression ratio for single HC21

Table 1 *Genes with significant over- or underexpression in DS patients in comparison to normal subjects.* (Red/green ratio = normal/DS (n/DS) ratio is provided as a mean of the four experiments; threshold for overxpression in DS cells was <0.35 (0.35 = mean-1 standard deviation, of the 334-gene set); other genes with ratio above the threshold but with significant p-values are listed). t-test: equal variance assumed; rank test: exact two-tails method. HLA-DRB3, major histocompatibility complex, class II, DR beta 3; SOD1, superoxide dismutase 1; GABRG2, gamma-aminobutyric acid (GABA) A receptor, gamma 2

Gene	Location	n/DS	p-value t-test	p-value Rank (Wilcoxon) test
HLA-DRB3	6p21.3	0.07	(>0.059)	0.029
SOD1	21q22.11	0.26	0.056	0.029
GABRG2	5q31-q33	0.29	0.010	(>0.059)
CD74	5q32	0.32	(>0.059)	(>0.059)
UCHL1	4p14	0.34	0.030	(>0.059)
LAG3	12p13.32	0.49	0.011	0.029
RELA	11q13	0.46	0.011	0.029
RXRG	1q22-q23	0.36	0.012	0.029
ZNF238	1q44-qter	0.47	0.021	0.029
RSU1	10p13	0.37	0.023	0.029
DNAJC8	1	0.47	0.055	0.029
KCNAB2	1p36.3	0.56	0.059	0.029
KIAA0102	11cen-q12.1	0.54	0.013	(>0.059)
RGS19	20q 13.3	0.51	0.037	(>0.059)
TMP21	14q24.3	0.52	0.032	(>0.059)
CSTB	21q22.3	0.62	(>0.059)	0.029
EML1	14q32	0.60	(>0.059)	0.029

Table 2 *Normal/DS expression ratio for genes localized on the same chromosome.* (Mean and standard deviation (SD) of the four experiments conducted on two cases and two controls. The HC 21 genes were: *CSTB, SMT3H, SOD1, SON, UBE2G*)

Chromosome	No. of genes	Mean	SD
1	30	0.894	0.377
2	27	0.955	0.349
3	16	0.874	0.182
4	6	0.871	0.353
5	17	1.035	1.105
6	24	1.203	1.256
7	11	1.179	0.469
8	9	0.977	0.289
9	15	1.176	0.799
10	11	0.955	0.501
11	24	1.321	1.259
12	21	0.857	0.258
13	7	0.976	0.160
14	10	0.926	0.264
15	3	1.832	1.925
16	13	0.886	0.311
17	25	1.044	0.389
18	3	1.268	0.780
19	24	1.010	0.484
20	8	0.776	0.208
21	5	**0.697**	0.274
22	9	0.850	0.264
X	16	1.019	0.450
Total set	334	1.015	0.670

genes, with at least two out of four experiments meeting the above described criteria, is shown in Table 3. This data further confirms a global overexpression of chromosome 21 genes, localized in the 21q22 region, in trisomic cells.

Cluster analysis using the 334 spots set, as defined above, was performed by hierarchical clustering (average linkage clustering) (Quackenbush, 2001) and indicated systematic differences among the various experiments, without discrimination between normal and DS subjects (Fig. 3A). When only HC21 genes (see Table 3) expression values were considered, expression data from DS patients tended to cluster separately to that from normal subjects (Fig. 3B).

Discussion

The 'cDNA array' methodology has come to be regarded as a powerful tool for the simultaneous analysis of mRNA expression levels of a large number of genes. The use of this technology in human diseases characterized by alterations in the genome structure is therefore justified. To date, very few reports have focused on gene expression profiles in human aneuploid cells. In such diseases it should be possible to systematically test hypotheses concerning 'gene dosage' effects due to replication or loss of wide genomic regions.

DS is a biomedical problem particularly suited to the simultaneous study of gene expression, since it is commonly hypothesized that the Down's phenotype arises from the overexpression of genes localized on HC21,

Table 3 Normal/DS expression ratio for all genes localized on chromosome 21 presents in MicroMax microarray. Exp. = experiment.

GenBank#	Gene name	Band	Exp. 1	Exp. 4	Exp. 2	Exp. 3	Exp. 1, 4, 2, 3
X02317	*SOD1* (Superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult)	21q22.11	0.18	0.30	0.30	0.24	–
X63071	*SON* (SON DNA binding protein)	21q22.11	0.88	0.58	0.97	1.01	–
AB002803	*BACH1* (BTB and CNC homology 1, basic leucine zipper transcription factor 1)	21q22.11	0.52	0.61	–	–	–
D84296	*TTC3* (Tetratricopeptide repeat domain 3)	21q22.2	0.16	0.13	0.15	–	–
X99584	*SMT3H* (SMT3 (suppressor of mif two 3, yeast) homolog 1)	21q22.3	1.19	0.96	0.80	0.29	–
AF032456	*UBE2G* (Ubiquitin-conjugating enzyme E2G 2 (homologous to yeast UBC7)	21q22.3	1.08	1.14	0.69	0.84	–
Z50022	*PTTG1* (Pituitary tumor-transforming 1 interacting protein)	21q22.3	0.33	0.58	–	0.41	–
L03558	*CSTB* (Cystatin B (stefin B)	21q22.3	0.59	0.70	0.39	0.80	–
X99209	*HRMT1* (HMT1 (hnRNP methyltransferase, S. cerevisiae)-like 1)	21q22.3	0.23	–	0.55	–	–
Mean			0.57	0.63	0.55	0.60	0.59
Standard deviation (SD)			0.37	0.30	0.27	0.30	–

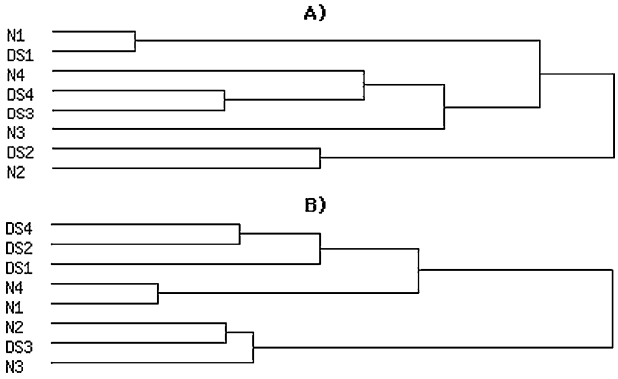

Figure 3 Expression data clustering: trees obtained by hierarchical clustering for data from experiments 1, 2, 3 and 4. A) Data concerning 334 genes from whole genome. B) Data concerning 9 genes localized on chromosome 21.

which is present in three copies in these patients. Several previous studies looked at the gene dosage effect for single genes (reviewed by Pritchard & Kola, 1999).

Our large-scale analysis of gene expression, using a 2400-probe slide, showed expression of about 50% of the studied genes at detectable levels. Data normalization was performed using a set of 34 housekeeping genes (glyceraldehyde-3-phosphate dehydrogenase - *GAPD*, beta actin, and 32 ribosomal proteins); remarkably, after this normalization, other housekeeping genes that were not considered for the transformation (such as the histone H3 and 'TATA binding protein' genes) turned out to be equally expressed in both cases and controls.

Genes with the highest levels of expression included many housekeeping genes, and most of the genes typically expressed in lymphocytes available on the array: prothymosin alpha, thymosin beta, thymosin beta 4, thymosin beta 10, retinoic acid alpha receptor, IL1 receptor, chemokine 6 receptor, 'NK cells transcript 4', macrophage inhibiting factor (*MIF*).

Searching for differential gene expression in normal versus trisomic cells, the most consistent result was the demonstration of the overexpression of superoxide dismutase mRNA (*SOD1*, at 21q22.11) and of four other genes in chromosomes other than 21. This finding was confirmed in both DS patients in two independent experiments. Interestingly, for the *SOD1* gene, a gene-dosage effect is well known for several DS cell types, including lymphocytes (first described by Feaster *et al.* 1977), and it is here confirmed as relevant in an open screening on thousands of genes, suggesting a specific role for the *SOD1* gene in the molecular physiopathology of DS T lymphocytes. It is commonly thought that the enhancement of SOD1 activity and of production of hydrogen peroxide in trisomy 21 cells, in presence of normal or low levels of reducing agents and enzymes involved in removal of hydrogen peroxide, may result in an excess of reactive oxygen and free radical damage. Although SOD1 activity was also reported to be enhanced in a DS brain (Brooksbank & Balazs, 1983), in a recent investigation on brains of control and DS fetuses

(Gulesserian *et al.* 2001) no detectable change was found in the expression of the SOD1 protein; this could imply a selective overexpression in specific cell types, such as lymphocytes. T cell deficiency has been repeatedly described in DS patients (for example, Cossarizza *et al.* 1990).

We also found a consistent overexpression of a particular major histocompatibility complex class II antigen coding gene, namely DR beta 3 (*HLA-DRB3*), in the DS lymphocytes; this finding could point to its possible role in the immune system defects typical of DS patients. Recently, a significantly lower frequency of "HLA-DRB blank" (non-DRB3/4/5) cells has been reported in patients suffering from rapidly progressive periodontitis; this is interesting, because early-onset periodontitis is a well known clinical problem associated with Down's syndrome (Cichon et al. 1998).

In addition, gamma-aminobutyric acid (GABA) A receptor gamma 2 (*GABRG2*), acetyl-coenzyme A acetyltransferase 2 (*ACAT2*) and ras suppressor protein 1 genes (*RSU1*) were found to be consistently overexpressed in DS T lymphocytes. Specific gene expression alteration in trisomic cells has recently been described in the literature as overexpression of several expressed sequence tags in placentas (Gross *et al.* 2002), focusing on possible diagnostic applications.

Although a possible factor affecting our results could be the difference in age between cases and controls, many studies about variation in gene expression with age, performed on individual genes or aimed at global gene expression analysis, suggest that variation, if present, affects a very small number of genes; hence, in absence of a positive proof that the expression of a particular gene is age-related, a relatively constant expression rate can be assumed for a specific cell type. Visala Rao *et al.* (2003) recently studied the influence of ageing on the gene-expression profile in human peripheral blood lymphocytes: the expression of a tiny minority of genes was found to be significantly altered in baseline conditions (76 out of 4052, in >70 years old subjects). *SOD1* was scrutined, and not considered significantly altered in basal conditions. Other studies have underlined that differences arise only when considering very large age ranges; in general, while senescence may affect the expression of some specific genes, many authors consider all subjects below 30-40 years as a homogeneous group with regard to gene expression. A very recent paper identified shared transcriptional profiles in aging across species; only a minority of specific gene categories showed significant expression variation patterns with age, and none of these categories included any gene that we here identify as overexpressed in trisomy 21 lymphocytes (McCarrol *et al.* 2004). Finally, no data about an age-related effect on expression can be found in the literature for any of the genes that we found to be the most overexpressed genes in trisomy 21 lymphocytes (*HLA-RB3*, *SOD1*, *GABRG2*). Taken together, these data tend to rule out a significant age-related component in the expression differences that we observe, although we cannot formally exclude an age-related or T-cell subset dependent contributing to the gene expression profiles.

When the data were clustered on the basis of chromosomal localization, the HC21 gene set showed on average the highest expression in DS cells in all the experiments (Table 2). The relevance of this observation, which did not include an *a priori* model, is clear regarding the presence of an extra copy of HC21 in trisomic cells; although based on a low number of samples, due to limited availability of human T lymphocytes in the required high amounts from DS patients, these findings reinforce the specific gene dosage theory in the pathogenesis of the DS phenotype, in contrast with the view of a massive global genomic disregulation due to aneuploidy (Pritchard & Kola, 1999). In addition, we failed to discriminate patients and controls on the basis of the 'genomic' gene set using cluster analysis, which considers the expression pattern for each gene and is not affected by the mean compensation effects on the genomic set, which are possible in the previous type of analysis. Conversely, a clear tendency for the grouping of patients and controls was obtained when restricted to HC21 gene expression values. In a recent transcriptome analysis of human trisomy 21 in fetal cells from pregnancies affected with trisomy 21 (FitzPatrick *et al.* 2002), the average level of transcription for the trisomic chromosome was found to be increased only by approximately 1.1-fold when compared to normal cells using array analysis. However, also in this study, grouping according to chromosomal location led to identification of the relevant trisomic chromosome as that with the most significant misregulation (both

chromosomes 21 and 13 in cells with trisomy 21 and trisomy 13, respectively).

Gene expression profile analysis in human T lymphocytes from patients with Down syndrome reinforces the specific gene dosage theory in the pathogenesis of the DS phenotype, and shows a consistent overexpression of the *SOD1* gene at 21q.

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