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Gene Expression Profile of Human Down Syndrome Leukocytes

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Aim Identification of differences in the gene expression patterns of Down syndrome and normal leukocytes.

Methods We constructed the first Down syndrome leukocyte serial analysis of gene expression (SAGE) library from a 28 year-old patient. This library was analyzed and compared with a normal leukocyte SAGE library using the eSAGE software. Reverse transcriptase polymerase chain reaction (RT-PCR) was used to validate the results.

Results We found that a large number of unidentified transcripts were overexpressed in Down syndrome leukocytes and some transcripts coding for growth factors (e.g. interleukin 8, IL-8), ribosomaproteins (e.g. L13a, L29, and L37), and transcription factors (e.g., Jun B, Jun D, and C/EBP beta) were underexpressed. The SAGE data were successfully validated for the genes IL-8, CXCR4, BCL2A1, L13a, L29, L37, and GTF3A using RT-PCR.

Conclusion Our analysis identified significant changes in the expression pattern of Down syndrome leukocytes compared with normal ones, including key regulators of growth and proliferation, ribosomal proteins, and a large number of overexpressed transcripts that were not matched in UniGene clusters and that may represent novel genes related to Down syndrome. This study offers a new insight into transcriptional changes in Down syndrome leukocytes and indicates candidate genes for further investigations into the molecular mechanism of Down syndrome pathology.

Down syndrome, caused by the trisomy of human chromosome 21 (HC21) is the most common congenital disease occurring in approximately 1 out of 700 live births. The most important of Down syndrome abnormalities are mental retardation, heightened risk of Alzheimer's disease, increased occurrence of leukemia, immunity and heart defects, and muscle hypotonia (1). In addition to the full HC21 trisomy, rare individuals with clinically recognized Down syndrome have only a partial trisomy (2). These cases allowed the determination of a region of about 4Mb located at 21q22-2-22-3 between the markers D21S17 and ETS2, termed Down syndrome critical region (DSCR) that, if triplicated, is presumably responsible for numerous Down syndrome features (3-6). Although this concept has dominated the field of Down syndrome research in the last few decades, Olson et al (7) have recently provided the evidence that the DSCR may not be sufficient to cause specific Down syndrome phenotypes. Also, patients with full trisomy 21 do not display all Down syndrome features, so analysis of more cases is necessary to clarify the contribution of 21q regions to the phenotypes of Down syndrome (2).

The generally accepted hypothesis is that HC21 contains genes that, if present in triplicate,

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contribute to the Down syndrome abnormal phenotype (8). Several studies were done to investigate potential alterations of gene product levels caused by an additional set of HC21 genes (9,10). Recently, it has been confirmed that Down syndrome fetal brain shows a global upregulation of HC21 genes in comparison with other chromosomes (11). These authors also found that there was a variable balance of expression levels in a set of upregulated HC21 genes, which may be responsible for the variations in cognitive clinical features among Down syndrome subjects.

Although this fact could probably occur in a variety of tissues, the molecular mechanism of the factors involved in the development of the complex Down syndrome phenotypes remains unclear. Assuming that the set of genes expressed in a tissue or cell type is responsible for the functional features displayed, a global analysis of gene expression would be highly relevant to generate the data helping to clarify the influence of several genes in the pathogenesis of Down syndrome.

Serial analysis of gene expression (SAGE, ref. 12) is an important research tool for increasing the extent of gene expression data (which can be analyzed simultaneously) from tens to thousands of transcripts. In SAGE, a short sequence tag derived from a specified position in an mRNA sequence is sufficient to uniquely identify a transcript. Once extracted from an mRNA population, the tags are concatenated, sequenced, and counted in order to measure the relative abundance of their corresponding transcripts. Although many SAGE tags can ambiguously match multiple known transcripts or have no match in the tag-transcript reference database and thereby restrict the amount of information, the technique has proved suitable for high-throughput screenings of expression profiles (13).

In this work, we used the SAGE technique to generate a comprehensive expression profile of Down syndrome leukocytes. We decided to study trisomic leukocytes because human blood is one of the few tissues that can be readily obtained from a patient by a non-traumatic procedure and public data from a normal leukocyte SAGE library was available for the purpose of comparisons. Here, we analyzed the differences in gene expression patterns between Down syndrome and normal leukocytes, and obtained useful information that could contribute to our understanding of how an altered expression of HC21 genes may lead to the development of undesirable features in Down syndrome leukocytes, such as the immune defects and the increased risks of leukemia and Alzheimer disease. The generated data could also help in finding novel genes as drug targets. For this purpose, we constructed the first-described Down syndrome SAGE library, determining a set of genes that are expressed differentially in Down syndrome leukocytes.

Materials and Methods

Samples

Venous blood samples were obtained from a 28-year-old male patient with Down syndrome from which the SAGE library was constructed and validated. Venous blood from a normal 28-year-old male subject was also collected for the purposes of validation. All the procedures and risks were explained verbally to the patient in the way that he could understand and to the normal individual, and a written consent form was signed by the normal individual and legal guardians of the Down syndrome patient. Total RNA was isolated from leukocytes using the "RNeasy Blood Mini Kit" (Qiagen, Santa Clarita, CA, USA) according to the manufacturer's recommendations.

SAGE

The SAGE library was generated using 5 μg of total RNA from Down syndrome leukocytes according to version B of the detailed instruction protocol of the I-SAGE Kit (Invitrogen, Carlsbad, CA, USA). The guidelines of the SAGE protocol have been outlined in a previous report (14). Recombinant pZErO-1 vectors (Invitrogen) were sequenced using the DYEnamic ET Terminator cycle sequencing kit (Amershan Biosciences, Piscataway, NJ, USA) in an ABI PRISM 377 DNA Sequencer (Perkin Elmer, Foster City, CA, USA). To improve the quality of Down syndrome sequence files before the tag count, the chromatograms were converted to PHD files and evaluated using Phred Phrap software, version 0.990722.g (*http://www.phrap.org/consed/consed.html#howToGet*). The eSAGE software (15) was used to qualitatively select, count, and catalogue the tag sequences. The minimal quality (Phred scores) to accept the tags' nucleotides was 15 for tag sequences and 12 for anchoring enzyme sites. Publicly available data from the normal leukocytes SAGE library of the

Duke University, Durham, NC, USA (*ftp://ftp.ncbi.nih.gov/pub/sage/seq/*) were used for gene expression comparisons with our Down syndrome leukocytes SAGE library. The "CGAP SAGE Genie" mapping data table (Hs.best_gene.gz) was obtained from the site *ftp://ftp1.nci.nih.gov/pub/SAGE/* and used for the identification of the sequenced tags from the two libraries. The comparisons, identification, and attributions of statistical values (*P* values) were also made by means of the eSAGE software. Some significantly differentially expressed transcripts were selected to validate the SAGE data, as candidates for involvement in Down syndrome, based on their descriptions and expression levels.

Reverse transcription (RT)-PCR Validation

The RT-PCR reactions were accomplished with the SuperScript II Reverse Transcriptase (Invitrogen), following the supplier's recommendations. Four hundred nanograms of total RNA were used for reverse transcription in a final volume of 100 µl using specific reverse primers from each selected transcript and from glycerol phosphate dehydrogenase (GPDH) as a control. The primer sequences used were as follows. Interleukin 8 (IL-8): sense 5'-ATg ACT TCC AAg CTg gCC gTg-3', antisense 5'- TTA TgA ATT CTC AgC CCT CTT CAA-3'; Chemokine C-X-C motif receptor 4 (CXCR4): sense 5'-ATG TCC ATT CCT TTG CCT CTT TTG C-3', antisense 5'-TTA GCT GGA GTG AAA ACT TGA AGA C-3'; BCL2-related protein A1 (BCL2A1): sense 5'-ATG ACA GAC TGT GAA TTT GGA TAT ATT-3', antisense 5'-TCA ACA GTA TTG CTT CAG GAG AG-3'; ribosomal protein L13a (RPL13a): sense 5'-GAA GGC ATC AAC ATT TCT GG-3', antisense 5'-TAA GAC CCT TTC CTT GCT CC-3'; ribosomal protein L29 (RPL29): sense 5'- CTT TCT CTT CCg gTT CTA gg - 3', antisense 5'- ACA AAT AgC ACA ggA ggA CC -3'; ribosomal protein L37 (RPL37): sense 5'-CTg CTA TAT CTT TCA CCA CC -3', antisense 5'-TTT gTC CAg TAA gTA CAg gg -3'; General transcription factor III A (GTF3A): sense 5'- gCg CCA ATT ACA gCA AAg CC -3', antisense 5'- gAC ATA CAT CCC TTT CTg gg -3'; GPDH: sense 5'-TGA AGG TCG GAG TCA ACG GAT TTG GT-3', antisense 5'-CAT GTG GGC CAT GAG GTC CAC CAC-3'.

To perform each subsequent PCR reaction, 2 µL of RT product was added in 0.2 mL micro tubes containing 200 µmol/L of each dNTP (Invitrogen); PCR buffer (Invitrogen) with 20 mmol/L Tris HCl (pH 8.4), 1.5 mmol/L $MgCl_2$ and 50 mmol/L KCl; 25 pmol of each primer and 1 unit of Taq DNA Polymerase (Invitrogen). The PCR protocol began with a heating temperature of 94°C for 1 minute followed by 35 cycles of 15 seconds at 94°C, 30 seconds at 55°C and 1 minute at 72°C. Three aliquots taken at different times during the amplification reaction were analyzed by PAGE and visualized by ethidium bromide staining, as previously described (16). The number of PCR cycles at which the aliquots were to be taken was optimized to 22, 25, and 28 for the GPDH control and 28, 30, and 35 for genes to be validated. This was due mainly to the low level of expression of the validated genes in relation to GPDH.

Results

We identified 31,643 SAGE tags representing 10,814 distinct transcripts. A list of all tags found is available on our web site (*http://www.ufscar.br/~dge/lbm.html*). In the control library there were 48,169 total tags and 15,046 unique tags. The distribution of unique tags in transcript copies per cell from the two libraries is summarized in Table 1. Most of the unique transcripts in the two libraries showed low level of expression; however, the categories with expression levels showing more than 10 copies per cell comprised more than 75% of the mRNA mass (Table 1). Ap-

Table 1. Distribution of unique tags in transcript copies per cell from the two libraries, according to their abundance and percentage of mRNA mass

	Down syndrome unique tags*		Normal unique tags	
Frequency	tag count (%)	mass fraction mRNA (%)	tag count (%)	percent mass fraction mRNA
More than 500	39 (0.36)	14.2	63 (0.42)	32.2
51-500	958 (8.86)	36.9	532 (3.53)	24.0
11-50	3,366 (31.13)	29.5	3,332 (22.15)	21.4
10 or fewer	6,451 (59.65)	19.4	11,119 (73.90)	22.4
Total	10,814 (100.00)	100.0	15,046 (100.00)	100.0

*Tag is a short sequence derived from a specified position in an mRNA sequence; it is sufficient to uniquely identify a transcript.

proximately 45% of the 10,814 unique tags of the Down syndrome library and approximately 27% of the 15,046 unique tags of the control library did not match UniGene clusters. A comparison between non-matched tags from the two libraries revealed that a significant fraction of Down syndrome nonmatches were represented by tags that appeared twice or more, reducing the probability of sequencing errors (Fig. 1).

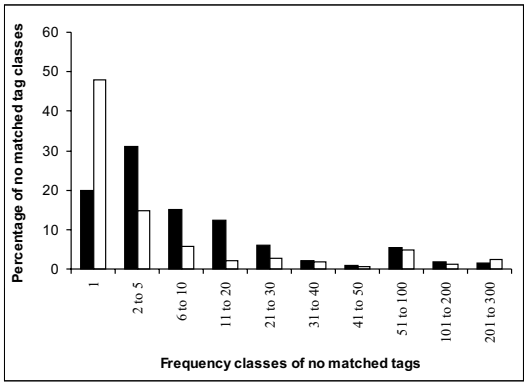

Figure 1. Distribution of no matched tags in Down syndrome and Normal libraries. A large number of non-matched tags was observed in the Down syndrome leukocyte SAGE library. Closed bars: Down syndrome library; open bars: normal library.

The expression profiles of normal and Down syndrome leukocytes were compared by classifying the top 120 highly expressed genes which showed high levels of significance from each library (Fig. 2). Genes related to the immune system, metabolism, and ribosomal proteins were predominantly expressed in normal leukocytes. On the other hand, a large number of unidentified transcripts were expressed in Down syndrome leukocytes (Fig. 2). The top forty overexpressed and the top forty underexpressed Down syndrome leukocyte transcripts are listed in Tables 2 and 3, respectively. Table 2 shows a significant number of Down syndrome transcripts that had no match in UniGene clusters, as discussed above. The normal library showed a large number of transcripts coding for hemoglobin (not shown in Table 3), which were not considered for further analysis.

Among the overexpressed genes observed in the Down syndrome library there was phosphoglycerate kinase I (PGK), an enzyme that plays an important role in the glycolytic pathway. Two genes involved in microtubule cytokinetics,

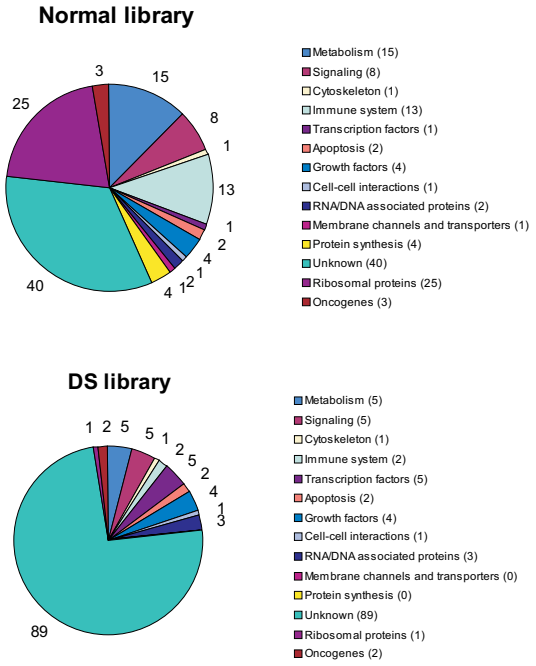

Figure 2. Functional classification of normal (upper panel) and Down syndrome (lower panel) leukocyte cells. A large number of unidentified transcripts were expressed in Down syndrome leukocytes. Genes related to the immune system, metabolism, signaling and ribosomal proteins were predominantly expressed in normal leukocytes. The number next to each category indicates the total number of genes in that class.

M-phase phosphoprotein I (MPP1) and ninein, were also overexpressed.

A number of transcripts coding for proteins engaged in processes that regulate the expression of several genes were significantly underexpressed in Down syndrome leukocytes. These included a regulator of chromatin related to SWI/SNF, the CCAAT/enhancer binding protein (C/EBP) beta, BCL2A1, IL8, Jun D proto-oncogene, and Jun B (Table 3).

The presence of three copies of chromosome 21 in the Down syndrome patient should lead to about 1.5-fold increase in the expression in comparison with the normal level of HC21 genes. The analysis of the expression levels of some genes from HC21 in normal × Down syndrome leukocytes showed that 6 out of 10 genes were overexpressed: NDUFV3 (DS6:N0), OLIG2 (DS6:N0), COL6A2 (DS5:N0), C21orf18 (DS5:N0), PTTG1IP (DS4:N0), C21orf80 (DS3:N0). Four were underexpressed: c21orf7 (DS0:N15), CNN2

Table 2. Comparison of Down syndrome (DS) and normal leukocyte transcript profiles – top 40 overexpressed Down syndrome leukocyte transcripts

Tag sequence*	Tag count		Description	UniGene (Hs. No.)†
	normal	DS		
TTTTTTTAGG	0	273	m-phase phosphoprotein 1	240
TGTACTTGGT	0	236	no match	
AGAGCAGAGA	0	217	zinc finger protein, subfamily 1A, 4 (Eos)	388622
GGAGCGACGT	0	178	no match	
GATTTATGCC	0	165	hypothetical protein MGC26694	303669
GCCGAGCGAA	0	119	no match	
CCTCTGCCAG	0	107	developmental pluripotency associated 4	317659
TGTGTATGTG	0	93	hypothetical protein FLJ13511	528701
CACACGCGTC	0	93	no match	
CTCAGGGACT	0	80	no match	
CACGACTGTG	0	79	no match	
GAACAGAAGA	0	79	acyl-coenzyme A dehydrogenase, short/branched chain	81934
AAGAATCTTG	0	79	no match	
AAGAGGCGCT	0	79	myosin light chain kinase 2, skeletal muscle	86092
CACCCGCGTA	0	78	no match	
TGTGTTAGCG	0	72	no match	
TGGTAAACAA	0	61	no match	
CCGTTCAACG	0	59	no match	
ATGGCGATGG	0	58	no match	
GGGCGCCGGA	0	57	paired immunoglobulin-like receptor beta	349256
GGCGGAGCAC	0	55	no match	
TGGTGTGTGG	0	52	no match	
GAGGAACGAA	1	52	ninein (GSK3B interacting protein)	385985
CGCATTGCAC	0	51	no match	
ATTTGAAGCT	0	48	no match	
AAAAAAGACA	0	47	dnaJ (Hsp40) homolog, subfamily A, member 3	6216
CAAACATCCA	0	46	no match	
TGATGGATGC	0	44	no match	
AAAATCAACA	0	42	general transcription factor IIIA	445977
TACGCCTGCC	0	41	no match	
CTTCGGCTTT	0	40	no match	
CGCAAAAACA	0	39	no match	
TGTCGTGGAG	0	38	ribosomal protein L4	186350
CCACGCAGAG	0	37	no match	
TGGAGGACGA	0	36	phosphoglycerate kinase 1	78771
CTAGTGGTCC	0	35	no match	
TTGCTGCTGA	0	35	pyridoxine-5'-phosphate oxidase	267963
TGGGAAAATA	0	34	cyclin D binding myb-like transcription factor 1	5671
CTACCATTGG	0	33	no match	
ACGGGCCGCA	0	33	no match	

*Tag is a short sequence derived from a specified position in an mRNA sequence; it is sufficient to uniquely identify a transcript. The tag sequences here represent the SAGE tags.
†UniGene matches and accession numbers (Hs. No.) are listed.

(DS0:N9), U2AF1 (DS0:N8), and ITGB2 (DS1:N10) in the Down syndrome library.

We also examined the expression of some ribosomal protein genes and found that most of them were underexpressed in Down syndrome leukocytes (Fig. 2). The expression level of three of these genes (L13a, L29 and L37) was also validated through RT-PCR, which was performed for the associated transcripts of some tags that showed significant differences in the level of expression. We selected and validated seven genes, including three ribosomal protein genes, with the same RNA sample used to build the Down syndrome SAGE library. An RNA sample obtained from normal leukocytes was used as a control. Consistent with the data obtained by SAGE, the expression levels of IL-8, CXCR4, BCL2A1 and the ribosomal proteins L13a, L29 and L37 were lower in Down syndrome leukocytes. On the other hand, the expression level of GTF3A was increased in Down syndrome (Fig. 3). These results were confirmed in triplicate, and by using RNA obtained from another Down syndrome patients (data not shown).

Discussion

Down syndrome phenotypes are ultimately a consequence of the disruption of genetic homeostasis caused by the presence of an additional chromosome 21. Several studies were previously carried out to analyze the gene dosage effect of single HC21 genes (17). An alternative to approaches that focus on unique genes is the use of experimental techniques that allow a large-scale analysis of gene expression, such as DNA micro-

Table 3. Comparison of Down syndrome (DS) and normal leukocyte transcript profiles – top 40 underexpressed Down syndrome leukocyte transcripts

	Tag count			
Tag sequence*	normal	DS	Description	UniGene (Hs. No.)†
TGGAAGCACT	198	0	interleukin 8	624
TCTCCATACC	140	0	no match	
GTGCGCTGAG	98	1	major histocompatibility complex, class I, A	181244
GTGCGCTGAG	98	1	major histocompatibility complex, class I, C	277477
TAACAGCCAG	86	0	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	81328
CCCTGGGTTC	164	2	ferritin, light polypeptide	111334
TCAAGAAAGT	81	0	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 1	172280
TCCAACGCGC	76	0	no match	
GCCAACCTCC	74	0	ADG-90 protein	334897
GCTGAACGCG	73	1	CCAAT/enhancer binding protein (C/EBP), beta	99029
GACAAAAAAA	69	1	ribosomal protein S15a	343665
GTGTCTGTTT	57	0	homo sapiens cDNA FLJ31102 fis, cloneIMR322000010	35580
TTTGTTAAAA	53	1	HIF-1 responsive RTP801	111244
ATGGTGGGGG	51	0	zinc finger protein 36, C3H type, homolog (mouse)	343586
CAATAAACTG	49	0	putative translation initiation factor	150580
TTAAACTTAA	43	0	chemokine (C-X-C motif), receptor 4 (fusin)	89414
AATGAACAAT	42	1	ninjurin 1	11342
TAATGAATAA	41	0	BCL2A1-related protein	227817
ACCCACGTCA	41	0	jun B proto-oncogene	198951
ACCCACGTCA	41	0	potassium voltage-gated channel, shaker-related subfamily beta member 2	298184
GTAAGAAAGT	39	0	KIAA0077 protein	112396
TTTAACGGCC	154	4	no match	
AGAAATAAAG	38	1	adenosine monophosphate deaminase 2 (isoform L)	82927
CTTGACATAC	37	0	dual specificity phosphatase 1	171695
ACATTTCCAA	36	0	putative lymphocyte G0/G1 switch gene	95910
CTGATCTGTG	34	0	major histocompatibility complex, class I, B	77961
ACAAGAAAGT	34	0	serine/threonine kinase 13 (aurora/IPL1-like)	98338
CAAACATTCT	34	0	no match	
TTAAGACTTC	33	0	SH3-Domain GRB2-like endophilin B1	136309
CTCAACGCGC	33	0	no match	
CTGGGGTTTC	33	0	immunoglobulin superfamily, member 4	70337
TACAGTATGT	31	1	glutamate-ammonia ligase (glutamine synthase)	170171
GCTAGGTTTA	30	1	no match	
GGGCCCCCTG	30	1	glycophorin C (Gerbich blood group)	81994
TGACTGTGCT	29	0	neurogranin (protein kinase C substrate, RC3)	26944
ACCCCCCCGC	29	0	jun D proto-oncogene	2780
CAGTGAATGA	29	1	homo sapiens cDNA FLJ10214 fis, clone HEMBA1006530	296532
GCCATCCCCT	27	1	hypothetical protein FLJ12876	348389
GAAACATTCT	27	0	nuclear transcription factor Y, gamma	168157
TGTAAAGATT	25	1	cyclin L1	4859

*Tag is a short sequence derived from a specified position in an mRNA sequence; it is sufficient to uniquely identify a transcript. The tag sequences here represent the SAGE tags.
†UniGene matches and accession numbers (Hs. No.) are listed.

arrays or SAGE. DNA microarrays allow a high-throughput screening of expression patterns in a parallel fashion. This, however, remains an expensive approach (13). On the other hand, the SAGE technique is suitable for average-sized laboratories, allows a wide range of analyses, and the expression level of any gene can be compared with that of any other gene from many libraries of different sources and sizes (18). A major drawback of SAGE is that it analyzes only known genes and requires a reliable tag-transcript reference database to correctly interpret the generated data (19). Both techniques have been previously used for the characterization of the expression profiles of aneuploid cells (8,11,20-22). To our knowledge, this is the first report of a genome-wide analysis of gene expression in Down syndrome leukocytes using SAGE. The screening of a leukocyte SAGE library constructed from a Down syndrome patient revealed a large number of non-matched tags. Since the Down syndrome tags were qualitatively selected, this number probably is not a result of sequencing errors. Hashimoto et al also found a large number of non-matched unique transcripts in normal leukocytes (23). These authors performed SAGE on a variety of normal leukocyte cell types and found that about 47% tags had no match, attributing the significant number of non-matched tags to several possibilities, ie hnRNA-derived sequences, PCR amplification error, mRNA unknown splicing variant, random binding of oligo-dT to mRNA, except by the poly A tail during construction of the cDNA library, and rear mRNA (23). At present, we cannot define whether non-

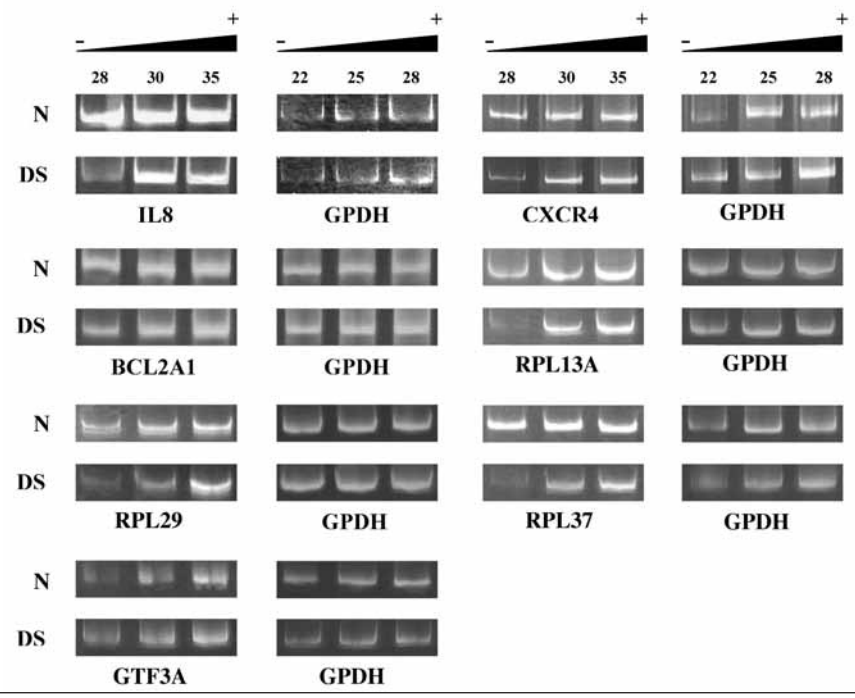

Figure 3. Assessment of quantitative accuracy of SAGE data by reverse transcription polymerase chain reaction (RT-PCR). Transcripts corresponding to seven genes were amplified semiquantitatively by RT-PCR and intensities of amplification products were compared to tag abundances in the respective SAGE library. The expression levels of the selected genes were relative to the number of SAGE tags: IL-8 (DS0:N198), CXCR4 (DS0:N43), BCL2A1 (DS0:N41), L13a (DS23:N126), L29 (DS37:N158), L37 (DS14:N177) and GTF3A (DS42:N0). Housekeeping gene transcripts (glycerol phosphate dehydrogenase, GPDH) are shown to the right of the specific gene transcripts. Similar results were found using RNA from other Down syndrome patients (data not shown). N – control (normal individual); DS – Down syndrome patient.

matched tags are real or not, but we suspect that the potential of SAGE to identify novel expressed genes (14) could permit the detection of as yet unidentified transcripts that appear in Down syndrome leukocytes. This may occur because rare or even inactivated transcripts under normal conditions could be overexpressed in Down syndrome leukocytes as a result of gene expression imbalance. This fact may, to some extent, explain the large number of non-matched tags found.

The gene coding for phosphoglycerate kinase (PGK) was overexpressed in our library. Interestingly, the gene is also overexpressed in the brains of Down syndrome patients and may be responsible for impaired glucose metabolism (24). M-phase phosphoprotein I (MPP1) and ninein were also overexpressed. MPP1 is a plus-end-directed molecular motor that binds to microtubules and is required for completion of cytokinesis (25). The human ninein was identified as a microtubule's minus-end-capping, centriole position, and anchoring protein required for targeting and regulating asymmetry centrosomes (26). The fact that these proteins were upregulated in Down syndrome leukocytes suggests that microtubule kinetics and, hence, cell division could be affected.

Some transcripts coding for proteins that affect the expression of several genes were significantly underexpressed in Down syndrome leukocytes. We assumed that low expression or absence of these proteins should lead to significant changes in the expression pattern, ultimately affecting the cells' normal behavior. These included a regulator of chromatin related to SWI/SNF that plays a role in facilitating transactivation of a variety of genes (27) and C/EBP beta, a transcription factor known to couple extracellular signal transduction pathways to numerous cellular processes that play a central role in lymphocyte differentiation (28). Genes coding for BCL2A1 and IL8 were also downregulated, indicating that regulation through NF-kappaB transcription factor is probably affected in Down syndrome leukocytes (29). In mice, the expression of BCL2 prosurvival homolog A1 protein is critical for the control of cell survival in B and T cells (30). In this context,

the underexpression of BCL2A1 in Down syndrome suggests its involvement in the impairment of lymphocyte proliferation. The genes c-jun, jun B, and jun D are members of the Jun family of transcription factors that are components of the activating protein-1 (AP-1) transcription factor (31,32), which converts extracellular signals into changes in the transcription of specific target genes involved in the control of cell growth and differentiation and neoplastic transformation (31). Jun B is able to regulate transcription in either positive or negative way, depending on the interacting partner and the promoter context (33). According to our SAGE data, Jun B and Jun D are downregulated in Down syndrome and this could be related to the impairment of DNA binding activity of AP-1 observed in Down syndrome leukocytes (34). Actually, the downregulation of IL8 observed in our library supports this observation, since IL8 expression is regulated primarily at the transcriptional level through cooperative interactions of the transcription factors NF-kappaB and AP-1 (35). Labudova et al reported that Jun D is also underexpressed in adult and fetal brain in Down syndrome, but not in Alzheimer disease, suggesting a role for impaired brain development (36). Interestingly, BCL2A1 and Jun B are also downregulated in children with acute lymphoblastic leukemia (37), and the expression of Jun B is inactivated by methylation in chronic myeloid leukemia (38). Whether these molecular changes are related to the increased risk of leukemia observed in Down syndrome remains a subject of further investigation and will require the analysis of additional patients.

Chemokines and their receptors play an important role in the site-directed migration and activation of leukocytes (39). Stromal cell-derived factor 1 alpha (SDF1 alpha) is a ligand of CXCR4 and is associated with G-protein-coupled signal transduction and leukocyte chemo-attraction (40). The CXCR4 are among the major receptors providing the triggering signals for B cell entry into lymph nodes (41). The underexpression of CXCR4 in Down syndrome patients may influence the emigration and activation of lymphocytes and ultimately affect immune functions.

As a whole, HC21 genes showed low level of expression in both Down syndrome and normal leukocytes and no significant differences in their expression levels were observed. Similar results were observed previously in the brains of mice with Down syndrome (8). Although we were unable to detect such differences with SAGE, we cannot conclude that there is no overexpression of HC21 genes in trisomic leukocytes, and the spatial and temporal expression pattern of these genes should be taken into account.

Several ribosomal protein genes were underexpressed in our library. Of note, Chrast et al (8) found a number of transcripts coding for ribosomal proteins showing underexpressed condition in the brains of mice with Down syndrome and in Down syndrome human amniocytes. Taken together, these findings indicate a possible pattern of subexpressed ribosomal protein transcripts in a variety of Down syndrome tissues. Since many ribosomal proteins have extraribosomal functions, including transcription, replication, RNA processing, DNA repair, and regulation of development (42), the functional consequences of this downregulation may not only be restricted to the ribosome and protein synthesis but may also affect other cellular processes. Current evidence indicates that lymphocytes show a reduction in the activity of ribosomal genes with age in Down syndrome (43) and that the rRNA 28S:18S ratio is low in AD patients, which may be due to changes in the regulation and expression of these genes (44). Together, downregulation of both rRNA and ribosomal protein genes in Down syndrome suggests an abnormal ribosomal biogenesis and consequently, an impaired translational activity in leukocytes and probably in other Down syndrome tissues. This, in turn, should lead to changes in gene expression.

In summary, we provided the first comprehensive leukocyte expression profile of a Down syndrome patient. This study identified significant changes in the expression pattern of Down syndrome leukocytes when compared with normal ones, including key regulators of growth and proliferation, ribosomal proteins, and a large number of overexpressed transcripts that had no match in UniGene clusters and that may represent novel genes related to Down syndrome. We should bear in mind that the differences in expression reported here might not be representative of all Down syndrome patients as the library was constructed using leukocytes from a single subject and there is probably biological variability among patients. Nevertheless, the RT-PCR analysis performed using RNA isolated from other patients allowed us to validate the

SAGE results for seven genes. The generated data can also be compared with that of other SAGE libraries constructed from different Down syndrome tissues in order to study the differences specific to temporal and spatial gene expression. Our library is currently being used to analyze the expression profile of Down syndrome leukemic patients in an attempt to clarify the relationship between trisomy 21 and the increased risk of leukemia observed in Down syndrome. The results reported here offer a new insight into transcriptional changes in Down syndrome leukocytes and indicate candidate genes for further investigations into understanding the molecular mechanism of Down syndrome pathology.

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