

ORIGINAL INVESTIGATION

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Gene expression patterns in cell lines from patients with 18q– syndrome

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Abstract Some studies have suggested that for trisomies, some genes are expressed far in excess of the expected 150% level and that this “dysregulation” is one of the mechanisms for the pathogenesis of trisomies. In an attempt to generalize this result to a monosomy, we examined mRNA isolated from lymphoblastoid cell lines derived from patients with 18q– syndrome, a deletion syndrome involving loss of the distal long arm of chromosome 18. Expression levels of ten chromosome 18 genes were compared between cell lines from eight patients with 18q– syndrome and four diploid controls. Gene expression was investigated by a quantitative reverse-transcription polymerase chain reaction (RT-PCR) method. With the exception of the transcription factor NFATC1, which shows a tendency towards gene dosage compensation (the expression pattern correlates with IgA deficiency), all of the other genes were expressed at a level proportional to their gene copy number. This was true regardless of mRNA abundance or different patterns of gene expression (ubiquitous versus tissue-specific gene expression). These results indicate that, unlike dysregulated gene expression apparent in some trisomies, this monosomic syndrome is largely due to consequences of reduced gene expression.

Introduction

Human aneuploidy results from the loss or duplication of specific chromosomes or chromosomal regions. Human 18q– syndrome results from deletions of the distal long

arm of chromosome 18. It was first identified by de Grouchy and colleagues (1964) and is a relatively common chromosomal deletion syndrome, with an estimated frequency of 1/40,000 live births. The extent of the 18q deletion varies from patient to patient, with most patients having terminal deletions, while a few have interstitial deletions (Chudley et al. 1992; Krasikov et al. 1992; Cody et al. 1997b). Phenotypic features vary from individual to individual. Some of the most common features include mental retardation, developmental delay, and dysmorphic facial features (Strathdee et al. 1995). Additionally, abnormally low levels or even the absence of serum and salivary immunoglobulin A (IgA) have been reported in some cases of 18q– syndrome (Hecht 1969; Stewart et al. 1970; Wilson et al. 1979).

The specific mechanisms underlying the molecular pathology of human aneuploidy remain unknown. One of the central hypotheses concerning molecular mechanisms of aneuploidy is the concept of the gene dosage effect, i.e., the amount of gene product is in proportion to the gene copy number (Junien et al. 1983). Evidence in support of the gene dosage effect has been accumulating (Epstein 1986). Virtually all of the gene products studied, however, have been basic metabolic enzymes, which are constitutively expressed. Little quantitative information is available on other tissue-specific genes such as transcription factors, receptors, and membrane components.

Alternative molecular mechanisms of aneuploidy have been proposed. These mechanisms include imprinting, as in Prader-Willi and Angelman's syndromes (Donlon 1988), gene dosage compensation, as in X-chromosomal genes (Forsdyke 1994), and gene dysregulation (Holtzman et al. 1992; Loughna et al. 1995). Using the trisomy 16 mouse, a murine model for human Down syndrome, Holtzman et al. (1992) studied the expression patterns of amyloid precursor protein (App) which maps to mouse chromosome 16. They determined that App was dysregulated (i.e., expressed at levels exceeding the 150% predicted by gene dosage) in various tissues of the trisomy 16 mouse.

Since there have been only limited reports on the molecular mechanisms of monosomic syndromes, we were

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interested in exploring the expression patterns of the haploid genes. In this study, we used lymphoblastoid cell lines as our model system to study the expression patterns of haploid genes because they are the only currently available resource from patients with 18q- syndrome. Lymphoblastoid cells from eight children with 18q- syndrome, each with different deletion breakpoints, were compared to cell lines from four chromosomally normal children. We analyzed these cell lines using ten different expressed sequence tags (ESTs) that map to chromosome 18q. The results of our study suggest that in monosomy most genes are expressed at their corresponding dosage level. The only exception was NFATC1, a T lymphocyte-specific transcription factor (Northrop et al. 1994). Instead of strictly obeying the gene dosage effect, NFATC1 was dosage-compensated in a subset of 18q- patients. Interestingly, this unique expression pattern correlates with the clinical IgA levels of the patients.

Materials and methods

Patient population

Patients with deletions of chromosome 18q were referred by their individual physicians or from the Chromosome 18 Registry and Research Society. The study was approved by the Institutional Review Board and informed consent was obtained from all subjects.

Lymphoblastoid cell lines and somatic cell hybrids

The lymphoblastoid cell lines were established previously as part of other studies (Cody et al. 1997b). In addition, somatic cell hybrids (human versus hamster) were constructed for many of the patients using selection for a locus on chromosome 18p. Each patient has at least two somatic cell hybrid cell lines. One contains an intact copy of chromosome 18 and the other contains the deleted copy. The method used to construct the somatic cell hybrid cell lines was described by Cody et al. (1997b).

Preparation of RNA samples

Lymphoblastoid cell lines were derived from four cytogenetically normal children (ranging from 3 weeks to 5 years old, two males and two females) and eight children with 18q- syndrome. Cell culture conditions were controlled in order to avoid RNA expression artifacts due to growth phase. Cell cultures were inoculated with a total of 2×10^7 cells and were expanded to 1.5×10^8 cells by adding 15 ml of fresh medium to each cell line every 2 days for 7 days. Fresh medium (10 ml) was added to each culture 6 h prior to harvesting. Total RNA samples from the lymphoblastoid cell lines were extracted according to the manufacturer's instructions using an RNA isolation kit (Genosys, Woodlands, Tex.). RNA samples were DNase I-treated (Promega, Madison, Wis.) and further purified twice by acid phenol-chloroform-isoamyl alcohol (25:24:1) extraction. RNA integrity was checked on a 1% agarose gel. Messenger RNA was isolated from the total RNA with the RNA Mini Isolation Kit (Qiagen, Chatsworth, Calif.).

Identification by RT-PCR of genes expressed in lymphoblastoid cells

Thirty-one 18q- region ESTs from a human EST gene map (Schuler et al. 1996) were screened by RT-PCR to identify genes that are expressed in lymphoblastoid cells. The mRNA sample

from one normal child was used as the template RNA for the screening. Primers of each gene were made according to the published sequence in the database. The EZ-buffer/DNA polymerase kit (Perkin Elmer, Norwalk, Conn.) was used for all RT-PCR reactions. RT-PCRs were performed with 25 ng of mRNA in a 25 μ l reaction volume. Each reaction consists of 100 nM of primers, 100 μ M dNTPs and 1 U of the DNA polymerase. Using a Perkin Elmer Model 9600 thermocycler, all RT-PCRs were done at 60 °C for 60 min, 94 °C for 1 min, followed by 30 cycles of two-step PCR with each cycle consisting of 94 °C, 30 s and 60 °C, 30 s and a final extension at 72 °C for 4 min. PCR products were examined on 2% agarose gels stained with ethidium bromide.

Genotypic analysis

Molecular analysis confirming the loss of genetic material and the extent of 18q loss was performed using PCR-based microsatellite markers as described by Cody et al. (1997b).

The ten genes expressed in lymphoblastoid cells were localized by PCR on the DNA of somatic cell hybrids derived from the eight patients with 18q- syndrome. Human genomic DNA was used as a positive control and the hamster DNA as negative control for each set of reactions. PCR was performed in a total reaction volume of 25 μ l, using 100 ng of genomic DNA, 200 nm of each primer, 100 μ M dNTPs and 0.25 U *Taq* polymerase with *Taq* Gold reaction buffer (Perkin Elmer, Norwalk, Conn.). All PCRs were first denatured at 94 °C for 4 min, followed by 30 cycles of amplification consisting of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C. PCR products were separated on a 2% agarose gel and stained with ethidium bromide.

Northern blot analysis

Human Multiple Tissue Northern I membranes (MTN, Clontech, Palo Alto, Calif.), which do not include lymphoblastoid cell RNA, were used to determine tissue expression patterns for each of the ten ESTs studied. For gene expression analysis, a northern blot was made using approximately 2 μ g mRNA from two controls and four children with 18q- syndrome (Maniatis et al. 1989).

Corresponding clones for the ten ESTs expressed in lymphoblastoid cells were obtained from Research Genetics (Huntsville, Ala.) (Table 1). The bacteria clones were streaked on LB plates containing 25 μ g/ml ampicillin. Colonies were amplified by touch PCRs using vector specific primers and the PCR products were purified (Qiagen, Chatsworth, Calif.). The purified PCR products were labeled with α [³²P] by random priming and the probes were purified on G-50 columns (5'-3', Boulder, Colo.). A total of 1×10^6 cpm/ml was used for the hybridization. Hybridizations were performed in ExpressHyb Solutions (Clontech, Palo Alto, Calif.) at 68 °C in a hybridization oven (Hybaid, Holbrook, N.Y.) overnight, and the MTN membranes were washed according to the manufacturer's instructions. MTN membranes were visualized using Kodak XAR-5 film. The northern blot membrane for gene expression analysis was visualized with a phosphorimager screen (Molecular Dynamics, Sunnyvale, Calif.) and the digitalized data was analyzed with Image-Quant Software (Molecular Dynamics). The MTN membrane probes were removed by boiling in a 0.5% SDS solution. The northern membranes were used up to four times.

Relative quantitative RT-PCR

The QuantumRNA Module (Ambion, Austin, Tex.) was used for relative quantitative RT-PCR. Titration of total RNA template determined a linear range of PCR amplification for each gene. The internal control was 18S rRNA. Different ratios of primers and a "competimer" for 18S rRNA were used to determine a linear range and a suitable amplification yield for the 18S control band in each assay. RT-PCRs with each primer pair of the ten genes expressed

in lymphoblastoid cells were performed on the total RNA samples. All RT-PCRs were performed in a 15 µl reaction volume, as described above. The 18S internal control primers did not work in the presence of the BCL2 and WI15134 primers, and the RT-PCRs for these two genes were, therefore, performed in parallel tubes rather than in concert.

The RT-PCR products were examined on agarose gels, stained with ethidium bromide, and destained in deionized water prior to quantitation. Quantitative analysis was performed using a digital system from IS100, Alpha-Innotech (San Leandro, Calif.). Relative expression levels were presented as ratios of the gene products to the 18S rRNA control.

Determination of IgA levels

IgA levels were determined at the Nichols Institute (San Juan Capistrano, Calif.). The laboratory provided age-appropriate ranges for normal levels of IgA. Standard deviations (SD) were estimated as one quarter of the normal range for that age. Z scores were determined using the following formula:

$$Z = \frac{(X \text{ subject} - X \text{ published median} / \text{age})}{SD}$$

Table 1 18q- cDNA markers screened (total = 31) (+ positive expression in lymphoblastoid cells, TS tissue-specific expression, U ubiquitous expression, ND no detectable northern bands on the MTN blot)

Marker	Gene	Size (bp)	B cell	Clone ID	Northern blot pattern
stSG1497		251			
WI-15134		136	+	175106	TS
A006w04		186			
SGC31888		150	+	45920	TS
SGC30781		150			
WI-6554		227			
BDY31g08		114	+	71839	TS
stSG8555		182			
SHGC31007		130			
StSG3734		149			
SGC32075		150	+	171880	U
KIAA0222		130	+	267742	U
SGC30835		125			
WI-10136		259			
U04313		186			
SHGC11871	NFATC1	231	+		TS
WI-7061		338			
SGC32555		150			
SGC9645		166			
WI-14125		126			
L40377	Antithrombin III	127	+	341978	U
SGC35852		123			
SGC33192		126			
A008M25		138			
SHGC12822		330			
A008V41		160	+	120013	U
WI-15059		125			
A008P37		145			
A007H41		131			
SGC33881		150	+	122709	ND
WI-7118	BCL2	333	+		U

Results

Screening and characterization of the ESTs expressed in lymphoblastoid cells

Using RT-PCR, we analyzed 31 ESTs which map to the distal part of chromosome 18q on a human EST gene map (Schuler et al. 1996). Of the 31 ESTs screened by the RT-PCR approach, ten were expressed in lymphoblastoid cell lines (Table 1). These ten ESTs were utilized for gene expression analysis of the lymphoblastoid cell lines from the eight patients with 18q- syndrome and the four diploid controls.

Genotypic analysis of the eight 18q- syndrome patients

The breakpoint analysis to define the extent of the deletion in the patients with 18q- syndrome utilized polymorphic markers mapped to the distal region of chromosome 18q as described by Cody et al. (1997b). Among the eight patients with 18q- syndrome, six have terminal deletions and two have interstitial deletions (Fig. 1). Somatic cell hybrids containing the deleted chromosome 18 from each

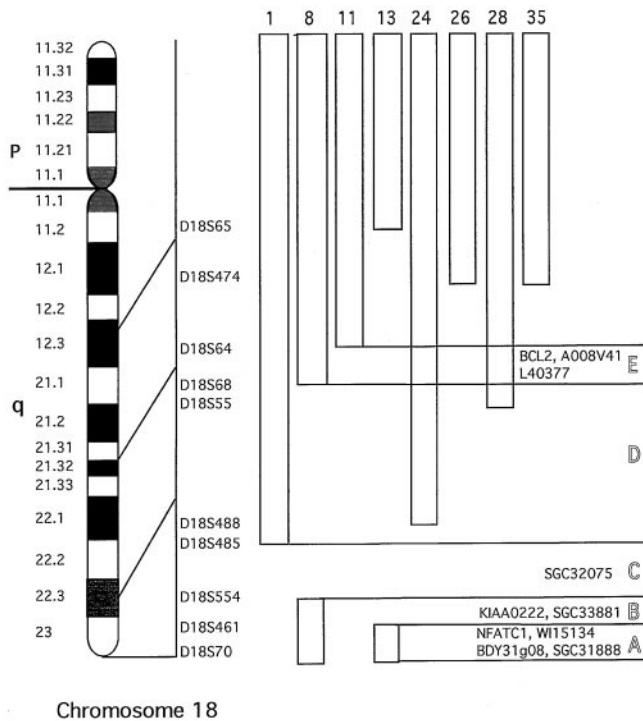


Fig. 1 Schematic representation of breakpoint analysis in 18q- syndrome patients. *Rectangles* represent deleted 18q of 8 patients. Subregions A, B, C, D, and E are designated. The DNA markers are placed in the order of the Genethon Linkage Map (Gyapay et al. 1994). The deletion breakpoints establish the copy number of each marker in individual patients (Cody et al. 1997b)

of the eight 18q- syndrome patients were used to map the ten genes by PCR. All ten ESTs were mapped to various parts of the 18q- region in agreement with the published data. We further divided the 18q- region into 5 subregions, A through E, based on the patients' deletion breakpoint data. ESTs for NFATC1, WI15134, BDY31g08, and SGC31888 mapped to subregion A; ESTs for KIAA0222 and SGC33881 mapped to subregion B; and EST SGC32075 mapped to subregion C, which is deleted in all of the patients. ESTs for BCL2, A008V41, and L40377 mapped to subregion E. None mapped to subregion D (Fig. 1).

Determination of PCR linear range amplifications of the candidate genes

The PCR linear range of all ten expressed genes was defined at 30 PCR cycles by titration of the template total RNA samples. Based on the relative abundance for each candidate gene EST, different starting concentrations of template RNA were used. The expression levels for the ten 18q genes ranged approximately 20-fold, with BCL2 and L40377 being the most abundantly expressed, and WI15134 being the least abundantly expressed transcripts (data not shown).

Characterization of tissue expression patterns of the candidate genes by northern blot analysis

We performed northern blot analysis using MTN blots to characterize the tissue expression patterns of the candidate genes/ESTs (Table 1). The MTN blot includes RNA from eight different human tissues: heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. BDY31g08, SGC31888, and WI15134 showed tissue-specific expression patterns. BDY31g08 is expressed in heart, skeletal muscle and pancreas. SGC31888 showed a strong band in placenta and two weak and different-sized bands in both heart and skeletal muscle. WI15134 is expressed in brain, but barely detectable in kidney and pancreas. There was no band detected for SGC33881 in the eight different tissues on the MTN blot. Therefore, the pattern of expression of SGC33881 is probably lymphoid-specific. ESTs for L40377, SGC32075, KIAA0222, A008V41 are expressed in all eight tissues and, therefore, are considered ubiquitously expressed.

NFATC1 is a known lymphocyte-specific transcription factor (Northrop et al. 1994; Venkataraman et al. 1994) and therefore is considered to be tissue-specific. BCL2 was cloned from B cell leukemia cells (Pegoraro et al. 1984; Tsujimoto et al. 1985). It is listed as ubiquitous in Table 1 because endogenous expression of BCL2 has been detected in a wide variety of tissue types (Nakayama et al. 1994).

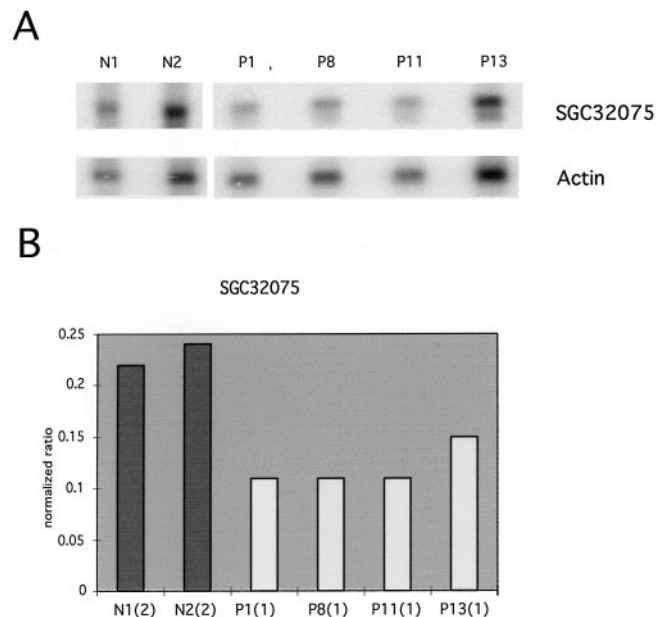
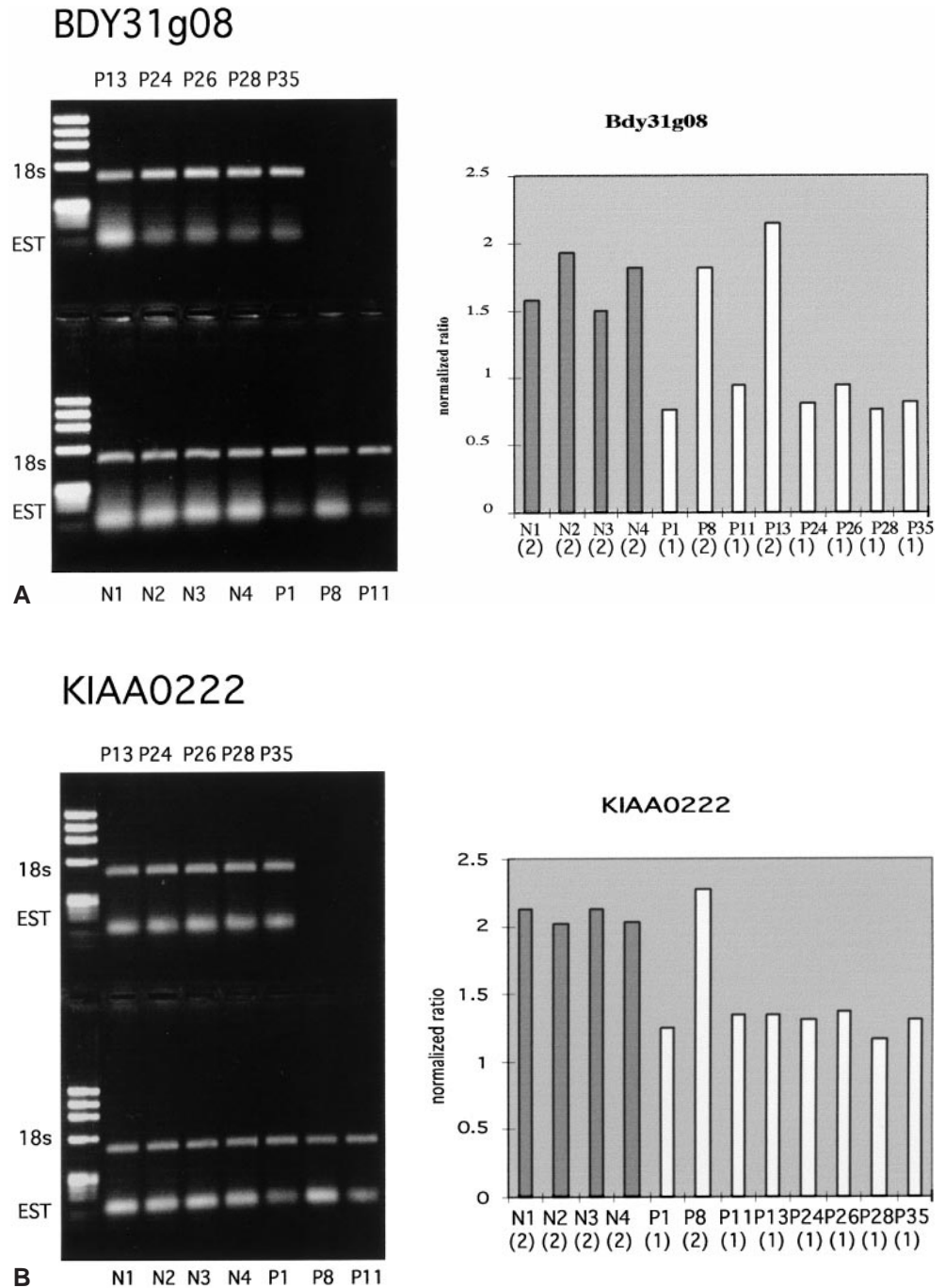


Fig. 2 A, B Northern blot analysis by EST SGC32075. **A** Autoradiographs of SGC32075 (*above*) and actin loading control (*below*) (*N* cytogenetically normal individual, *P* 18q- syndrome patient). **B** Histogram showing expression levels of the EST normalized to actin loading control. Copy number of the EST in the samples is shown in *parentheses*. The result is consistent with the quantitative RT-PCR of SGC32075 shown in Fig. 3c

Fig. 3A–D Relative quantitative RT-PCR of four ESTs. The *left part* of each figure is the actual agarose gel image. In each, 12 samples are loaded in the *upper and lower panels* of the same gel. *Lane 1* is the ϕ X174 DNA marker. The *upper band* in the remaining lanes is the 488 bp 18S rRNA control and the *lower band* is the EST. The histograms on the *right* show the expression levels represented as normalized ratios of the EST products to the 18S rRNA controls. *Numbers in parentheses* denote the copy numbers of the ESTs in the samples. **A** BDY31g08. **B** KIAA0222. **C** SGC32075. **D** L40377. (*EST candidate gene, N normal individual, P 18q- syndrome patient*)



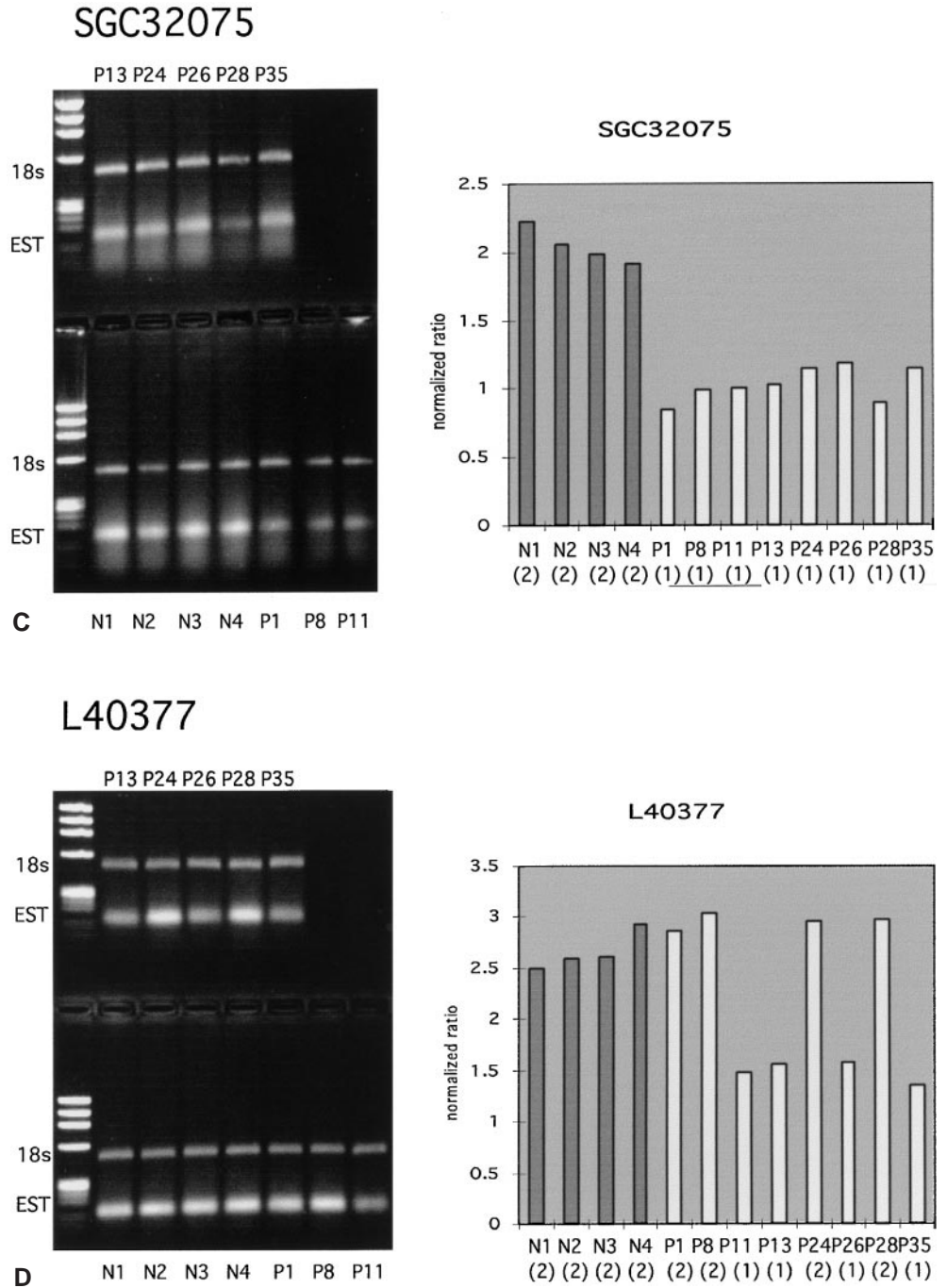
Relative quantitative RT-PCR and northern blot analysis of candidate gene expression

Northern blot analysis was performed in parallel with the relative quantitative RT-PCR for SGC32075. The results of these experiments were completely consistent with each other (Figs. 2 and 3c). The relative quantitative RT-PCR method was used to define the relative expression levels of the other nine ESTs in the eight 18q- syndrome patients and four controls. The results for four ESTs representing each subgroup are illustrated in Fig. 3.

All but one of the ten ESTs were expressed according to the corresponding gene dosage in the eight 18q- syndrome patients. The exception was NFATC1 in patients No. 26 and No. 35. Instead of expressing at half the normal level, NFATC1 was dosage-compensated yielding diploid levels of expression. In patient No. 1, NFATC1 also showed a tendency toward gene dosage compensation, and was expressed at close to the normal diploid level. In patient No. 11, NFATC1 expression was reduced to slightly higher than the half dosage level (Fig. 4).

This quantitative RT-PCR expression data was highly reproducible for at least three independent sets of experi-

Fig.3 C-D



ments performed for all ten genes (with each sample showing the corresponding dosage level expression), with the exception of NFATC1. The results for NFATC1 were repeated five times.

Determination of IgA levels

IgA levels were measured clinically in seven of the 18q-syndrome patients, and the results are summarized in Table 2. Two patients lacking deletions (Nos. 8 and 13) had normal NFATC1 and IgA levels. Three patients with NFATC1 deletions (Nos. 1, 26, and 35) showed NFATC1

dosage compensation, and had normal IgA levels. Two other NFATC1 deletion patients (Nos. 11 and 28) showed reduced NFATC1 mRNA levels, and had abnormally low IgA levels. Another NFATC1 deletion patient (No. 24) also showed low NFATC1 levels, but the corresponding IgA level could not be determined because of blood sample hemolysis.

Discussion

Human aneuploidy is characterized by a variety of phenotypic abnormalities as a result of imbalanced gene

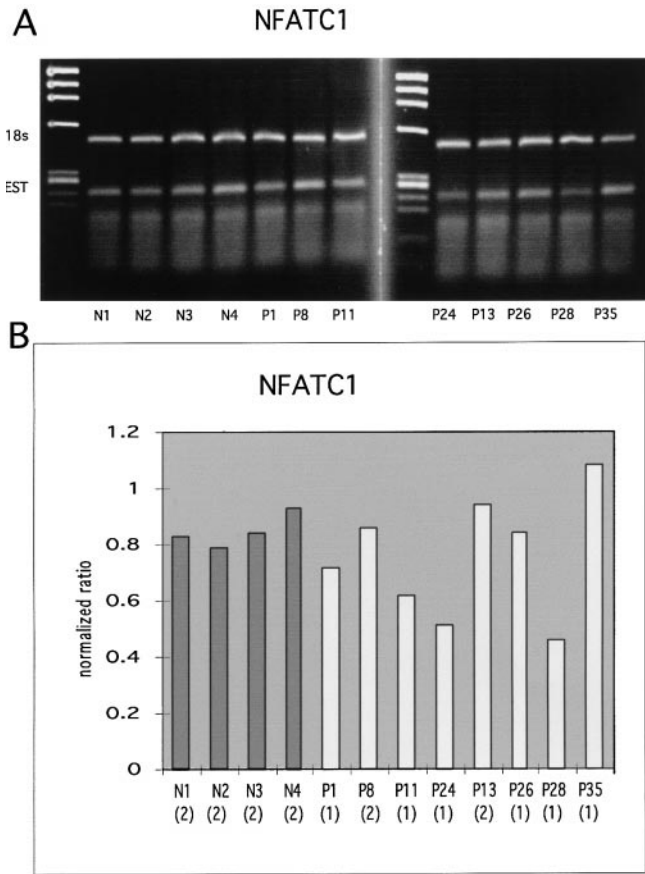


Fig. 4A, B Expression pattern of NFATC1. **A** The 12 samples were loaded onto two agarose gels of the same concentration. Lane 1 is the ϕ X174 DNA marker. The upper band of both gels is the 488 bp 18S rRNA control and the lower band is NFATC1. **B** The expression levels are represented as normalized ratios of NFATC1 to the 18S rRNA controls on the Y axis. Numbers in parenthesis denote the copy numbers of the gene in each corresponding sample. (N cytogenetically normal individual, P 18q-syndrome patient)

Table 2 IgA levels and NFATC1 expression (DC dosage compensation, NA not available)

Patient number	IgA	Z score	Diagnosis	NFATC1 level
1	152	-0.7	Normal	Normal (DC)
8	162	1.1	Normal	Normal
11	< 8	< -3.0	Deficient	Reduced
13	207	-1.2	Normal	Normal
24	NA	NA	NA	Reduced
26	265	3.3	Normal	Normal (DC)
28	16	-2.5	Low	Reduced
35	134	1.1	Normal	Normal (DC)

dosage. Some studies present evidence that dysregulated gene expression may play a role both in animal models of human trisomy and in human trisomic fetuses (Holtzman et al. 1992; Loughna et al. 1995). The purpose of this investigation was to examine another form of aneusomy, namely monosomy, for expression of genes located

within the deleted region. Our results suggest that in monosomy most genes are expressed at their corresponding dosage levels.

Expression of genes according to the gene dosage effect in chromosomal deletion syndromes could result in two possible outcomes, i.e., haploinsufficiency and haplo-sufficiency (Fisher and Scambler 1994). Haploinsufficiency (i.e., an abnormal phenotype resulting from deletion of one gene copy), is believed to be the principal mechanism for chromosomal deletion disorders (Wilkie 1994). There have been previous studies of genes mapped to the 18q- region which support the notion of haploinsufficiency. Magness et al. (1994) reported that half level expression of the FECH (ferrochelatase) gene in a patient with 18q- syndrome led to elevated levels of erythrocytic and fecal protoporphyrin, the diagnostic feature of EPP (erythropoietic protoporphyria). Gay et al. (1997) found incomplete myelination in patients with 18q- syndrome by magnetic resonance imaging, implying that the myelin basic protein (MBP) gene is haploinsufficient. MBP localizes within the 18q22-qter region (Saxe et al. 1985) and is a major protein component of the myelin sheath in the central nervous system (Matthieu 1993). Cody et al. (1997a) demonstrated decreased growth hormone levels in 18q- syndrome patients which might be caused by a decreased level of GALNR1 (galanin receptor 1). GALNR1 maps to a critical region of 18q, which is deleted in all patients with growth hormone insufficiency (Nicholl et al. 1995). Galanin is an important neuromodulator present in the brain, gastrointestinal system, and hypothalamo-pituitary axis. Galanin has been implicated in growth hormone secretion and many other biological responses (Bedecs et al. 1995). Based on their basic neuro-specific activities, there is no indication that MBP and GALNR1 are expressed in B lymphocytes. Therefore, we did not include MBP and GALNR1 as candidates in this gene expression study.

There are also cases of haploinsufficiency (i.e., deletion of one gene copy without any apparent abnormal phenotype; for example see Wilkie 1994). It is a non-disease-causing mechanism and might be a principal means by which patients tolerate chromosomal deletion syndromes. Kacser and Burns (1981) proposed a theory of metabolic fluxes to explain why many inborn errors of metabolism are recessive. Assuming that a metabolic pathway has many non-rate-limiting steps, control of flux at any particular point in a pathway will be small. Hence, many pathways show a saturable relationship between enzyme level and metabolic flux, with fluxes fully saturated at wild type enzyme levels. A 50% reduction in enzyme activity would therefore cause little reduction in flux below its saturation level. This theory is supported by Muller (1948) who found that the concentrations of most enzymes within diploid cells corresponded to a point well along the plateau of the dose-response curve. In most cases, halving the concentration of the enzyme would still generate a product level corresponding to the plateau of the dose-response curve (Forsdyke 1995). While various recessive mutations are good examples, the Peptidase A

(PEPA) gene, an 18q- specific gene, provides additional evidence for this theory. In carriers of recessive mutations for PEPA, half dosage levels of PEPA expression have no associated phenotype (Danesino et al. 1978).

Another alternative explanation for haplosufficiency is that the haploid genes either up-regulate their expression to compensate for lower copy number or act through some unknown mechanisms. Gene dosage compensation is characteristic of genes on X-chromosomes (Fordsdyke 1994). This phenomenon has also been observed in many lower organisms like prokaryotes, yeast, tomato and maize (Goldberger 1974; Osley and Hereford 1981; Fobes 1980; Birchler 1981). However, gene dosage compensation has never been known to occur in mammals. Our study has demonstrated that NFATC1 shows some degree of dosage compensation in some patients with 18q- syndrome. This finding highlights the possibility that a highly tissue-specific gene might be able to overcome haploid copy number through expression at the diploid level.

A subset of 18q- patients demonstrate IgA deficiencies, despite normal levels of B lymphocytes (Lewkonia et al. 1980). Immunoglobulin gene abnormalities can be ruled out, since the IgA heavy chain genes are located on chromosome 14 and the light chain genes are on chromosomes 2 and 22 (Hecht 1969). Therefore, IgA regulatory loci located within the 18q- region have been suggested to account for this defect (Stewart et al. 1970; Lewkonia et al. 1980). NFATC1 is known to be critical for interleukin 2 gene induction in activated T cells (Banerji et al. 1991). Venkataraman et al. (1994) reported that NFATC1 is also expressed in B cells. They showed that NFAT binding sites could activate transcription in PMA and ionomycin- or anti-immunoglobulin-stimulated Bal-17 cells, indicating that NFATC1 is a transcriptional activator. Our data suggests a relationship between NFATC1 gene expression and IgA levels in 18q- patients. These data imply that NFATC1 plays a role, either directly or indirectly, in the regulation of IgA expression.

An interesting finding regarding human aneuploidy is that, while many phenotypes are distinguishable, there is a striking overlap of the features, such as mental retardation and short stature. Most of these overlapping features are independent of the specific chromosomes affected or the extent of loss or gain of DNA. One school of thought regarding this observation is that regulatory disturbances are caused by imbalanced gene dosage, i.e., that aneuploidy could influence the expression of additional genes mapping to other chromosomes (Vogel 1973). In fact, in the studies of Holtzman et al. (1992), the gene for prion protein, although localized on mouse chromosome 2, was dysregulated in the brain tissues of the trisomy 16 mouse. Whether we can generalize this phenomenon to monosomies is the question for future studies.

In conclusion, the information gained from this study should help elucidate the molecular mechanisms of human monosomic syndromes. It suggests that haploinsufficiency may be the underlying cause of monosomic syndromes and that dysregulation may not be a common mechanism. Our findings regarding the expression pattern

of NFATC1 imply that some haploid genes can undergo dosage compensation by increasing their level of expression. The role of NFATC1 in the regulation of IgA levels in 18q- patients remains a subject for further exploration.

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