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# Direct Gene Dosage Determination in Patients with Unbalanced Chromosomal Aberrations Using Cloned DNA Sequences. Application to the Regional Assignment of the Gene for α2(I) Procollagen (COLIA2)

CLAUDINE JUNIEN,[1] CÉCILE HUERRE,[1] AND MARIE-ODILE RETHORÉ[2]


## SUMMARY

We describe a new method of direct gene dosage determination in patients with unbalanced chromosomal aberrations using cloned DNA sequences: the intensity of the signal obtained by hybridization of the radioactive probe to the corresponding DNA fragments can be compared with the intensity of the DNA fragments that hybridize with a nonsyntenic probe used as an internal control. This has been demonstrated by densitometer tracing of the autoradiogram, using an X-specific DNA sequence, β globin and α2(I) collagen probes, in normal men and women, in one patient trisomic for 11p, and in one patient trisomic for segment 7q21→7qter. The ratio *men/women* for the X-specific sequence (DXS) was close to the expected value 0.5, while the ratio *trisomy 11/normal control* and *trisomy 7/normal control* were close to 1.5 for β globin (HBB) and α2(I) collagen (COLIA2), respectively. The gene coding for COLIA2 can therefore be assigned to 7q21→7qter. This method should also apply to noncoding sequences: the increasing number of cloned DNA segments that have already been assigned to a specific chromosome represent a new tool for prenatal and premorbid diagnosis of unbalanced chromosomal aberrations.


## INTRODUCTION

Gene dosage studies in patients with chromosomal aberrations have provided the most precise regional chromosomal assignments for human genes. However, this

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[1] Institut de Pathologie et Biologie Cellulaires et Moleculaires, INSERM U 129, 24 rue du Fg St Jacques, 75674 Paris Cedex 14, France.

[2] Institut de Progénèse, 15 rue de l'Ecole de Médecine, 75270 Paris Cedex 06, France.

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method can be applied only to genes expressed in a limited number of human tissues: erythrocytes, leukocytes, fibroblasts, lymphoblastoid cell lines, and hair roots (for a review, see [1]). More recently, the use of cell lines with an abnormal number of X or Y chromosomes has also proven useful in assessing the identity of clones belonging to chromosome-specific libraries [2–5]. We therefore assumed that it should be possible to count the actual number of genes in aneusomic patients using specific cloned cDNA or genomic DNA as molecular hybridization probes.

However, in Southern blot experiments, the intensity of the hybridization signal for the same fragment may vary from one lane to another even with the same amount of DNA. It therefore appeared more suitable to compare, in the same lane, the intensity of a fragment corresponding to a specific DNA region with the intensity of a fragment corresponding to a nonsyntenic gene used as an internal control. This would overcome variations presumably due to differences in DNA transfer.

The aim of this study is twofold: (1) to test the accuracy of this method by comparing the relative intensity of the hybridization signal of an X-specific DNA segment (DXS) in normal men and women for the monosomic versus disomic state, and of a β-globin genomic probe in a patient trisomic for the chromosomal region (11p125→11p128) that carries the gene for β globin (HBB) [6, 7] and in normal individuals for the trisomic versus disomic state. In both instances, the internal control used was a cDNA for α2(I) collagen; (2) to refine the chromosomal assignment of the α2 chain of type I collagen (COLIA2) that we recently assigned to chromosome 7 [8] by comparing the relative intensity of the hybridization signal of α2(I) collagen in a patient trisomic for 7q21→7qter and in normal individuals using the β-globin probe as the internal control.

## MATERIALS AND METHODS

### *Patients*

Patient no. 1 is reported elsewhere [6, 7]. He is an adolescent trisomic for 11p with the exception of band 11p13. Patient no. 2 is an 8-month-old girl with trisomy 7q resulting from a paternal translocation t(3;7)(p26;q21). Her case will be reported elsewhere.

### *Karyotypes*

Karyotype analyses were performed after RHG and GTG banding. Prometaphases were obtained by thymidine synchronization, bromodeoxyuridine incorporation, and Hoechst 33258-Giemsa staining CRTBG banding [9]. Skin biopsies were obtained from the patients, and karyotyping of the fibroblasts was performed after RHG banding.

### *Description of Nucleic Acid Hybridization Probes*

The X-specific DNA segment was cloned in pBR322 and consisted of a 2.7-kb Eco RI genomic DNA fragment that maps to Xq13→Xq23. This probe was provided by J. L. Mandel.

The probe for β globin was obtained from T. Maniatis and A. Bank. This is the pBR322 plasmid containing the 4.4-kb human β-globin gene subcloned fragment [10].

The cDNA coding for α2(I) collagen was isolated and cloned in pBR322: this clone has been shown to contain a 2.2-kb insert coding for the region of the human proα2 chain of

type I collagen extending from amino acid position 450 to the middle of the C terminal propeptide [11].

The recombinant plasmids were nick-translated to a specific activity of $8 \times 10^8$ cmp/μg using a nick-translation kit and $\alpha^{32}$PdCTP purchased from Amersham International (Amersham, Buckinghamshire, England).

### *Southern Blot Experiments*

DNA was prepared from fibroblasts as described [12]. Aliquots of 30 μg of DNA were digested with restriction endonucleases according to the manufacturer's recommendations (Biolabs, Beverly, Mass.; Boehringer, Mannheim, West Germany). DNA was electrophoresed and then transferred to nitrocellulose as described [13]. Hybridization with the radioactive probes was carried out for 16 hrs using 10% dextran sulfate [14], as described [8]. Densitometer scanning profiles of the autoradiograms were obtained using a Gelman apparatus.

## RESULTS

In preliminary experiments, we studied the normal restriction pattern of human DNA digested with several restriction endonucleases and hybridized either separately with the three different probes (β globin, X-specific DNA, and α2(I) collagen) or with a mixture of two probes: (1) X-specific DNA plus α2(I) collagen and (2) β globin plus α2(I) collagen (data not shown). The restriction endonuclease Eco RI appeared to be the most suitable for comparing the intensity of the signals obtained for the DNA fragments that hybridized with the three different probes.

The densitometer scanning profiles of the autoradiograms corresponding to an Eco RI digest of normal DNA hybridized with a mixture of two different probes are shown in figure 1. Some fragments resolved as unique, well-separated peaks, while others resolved as overlapping bands, giving only an approximate idea of their relative heights, but allowing control of possible variations for different fragments within a lane (fig. 1*a* and *b*). Although the kinetics of hybridization

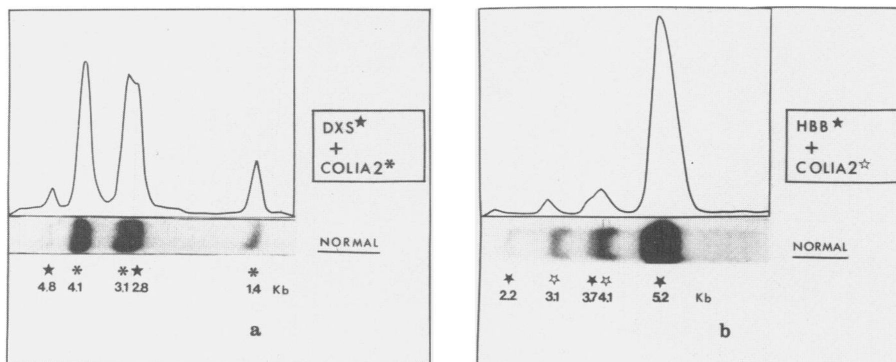


FIG. 1.—Comparison of the hybridization signal of DNA fragments hybridized with two different probes. Autoradiograms and densitometer scanning profiles of normal human DNA digested with endonuclease Eco RI and hybridized with a mixture of two radioactive probes, showing juxtaposition of specific DNA fragments related to both probes. *a*, X-specific DNA (DXS) + α2(I) collagen (COLIA2): 4.8-kb, 2.8-kb DXS fragments plus 4.1-kb, 3.1-kb, and 1.4-kb COLIA2 fragments (the 1.2-kb COLIA2 fragment is not shown). *b*, HBB + COLIA2: 5.2-kb, 2.7-kb, and 2.2-kb HBB fragments plus 4.1-kb and 3.1-kb COLIA2 fragments; the 1.4-kb and 1.2-kb COLIA2 fragments are not shown.

were not evaluated in this study, the comparison of peaks of different intensity clearly indicated that the concentration used for the three probes was not rate-limiting under the conditions described.

We therefore chose to compare the computed peak areas of: (1) the 4.8-kb fragment of DXS with the 1.4-kb fragment of COLIA2, and (2) the 2.2-kb fragment of HBB with the 3.1-kb fragment of COLIA2. Analysis of several traces in different lanes indicated that the ratio of the peak area of the 4.8-kb fragment (DXS) to the 1.4-kb fragment (COLIA2) was fairly constant for normal men (0.431 ± 0.074) and for normal women (0.789 ± 0.033). The mean value for men was thus approximately half the value obtained for women: 0.54 ± 0.11 (fig. 2*a*).

Likewise, the mean ratio of the peak areas of the 2.2-kb HBB to the 3.1-kb COLIA2 (0.495 ± 0.027) was significantly increased in patient no. 1 (trisomy 11p) when compared to the corresponding value of 0.366 ± 0.024 found in normal individuals. These values indicated an overall 35% ± 9% increase for HBB (fig. 2*b*).

We had the opportunity to study a patient trisomic for the distal two-thirds of the long arm of chromosome 7 (7q21→7qter). Peak areas for 3.1-kb COLIA2

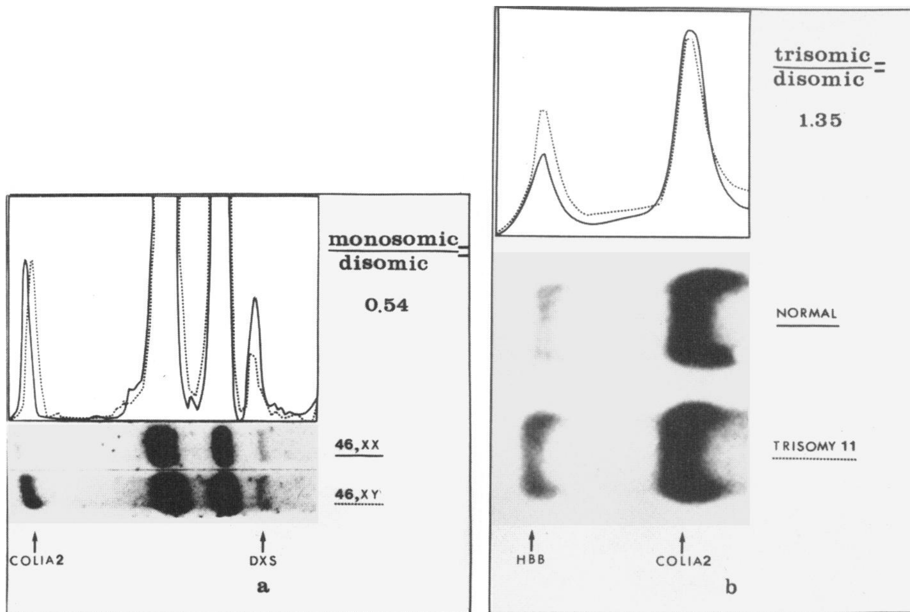


FIG. 2.—Comparison of monosomic, disomic, and trisomic states. Autoradiograms and densitometer scanning profiles of genomic DNA digested with endonuclease Eco RI and hybridized with a mixture of two probes. *a*, Comparison of the 4.8-kb DXS fragment with the 1.4-kb COLIA2 fragment in normal men (*dotted line*) and normal women (*full line*); *b*, comparison of the 2.2-kb HBB fragment with the 3.1-kb COLIA2 fragment in a normal individual and in patient no. 1 trisomic for the short arm of chromosome 11. Only well-separated peaks giving a similar hybridization signal that were retained for comparison of their relative intensity by densitometry are shown in this figure. The height of the peak used as an internal standard (i.e., COLIA2 in *a* and *b*) was adjusted to the same level in the different lanes to be compared by expanding the scale sensitivity of the Gelman apparatus. Areas under the peaks were calculated in several traces and in several lanes for the same individual.

and 2.2-kb HBB were compared. The mean ratio of the peak areas COLIA2/HBB was significantly higher for the trisomic patient (4.53 ± 0.53) than for normal controls (2.73 ± 0.16), that is, a 68% ± 28% increase (fig. 3).

These numbers are mean values of several traces in different lanes for the same individual(s) in the same experiment. The mean peak area ratio of the probe to the internal control may vary for the same individual from one experiment to another, possibly because of variations in specific activity. Because of this, peak areas were always compared on the same autoradiogram corresponding to the same experiment. Although these peak area ratios were confirmed in several experiments, the data given in the text refer only to one experiment and cannot be taken as mean values of several experiments.

## DISCUSSION

The increasing number of cloned DNA segments that have already been assigned to specific human chromosomes are an invaluable new tool for increasing our knowledge of the human gene map. Regional assignment of these segments can be achieved by in situ hybridization and/or using somatic cell hybrids with a balanced translocation involving the chromosome that carries the gene or the DNA segment. It should also be possible to use material from patients with unbalanced chromosomal aberrations provided a reliable and precise quantitative method is available. Using densitomer tracing of autoradiograms obtained by Southern blot experiments, we show that it is possible to measure accurately a half-normal value or a sesquialteral (one and one-half) value related to a monosomy

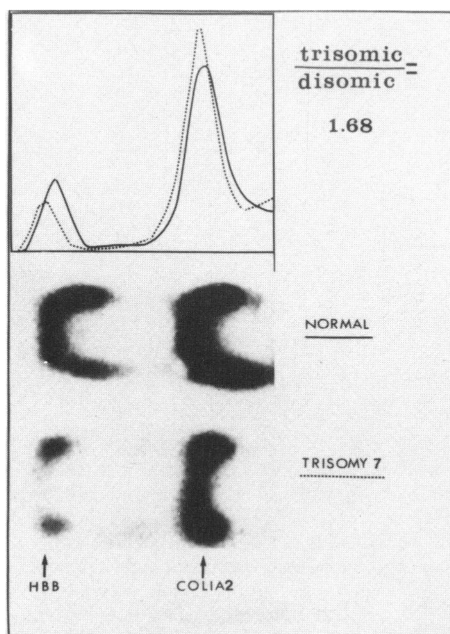


FIG. 3.—Regional assignment of COLIA2. Same experiment as in figure 2*b* showing comparison of a normal individual (*full line*) with patient no. 2 trisomic for segment 7q21→7qter (*dotted line*).

or a trisomy, respectively. For technical reasons, the intensity of the hybridization signal of the DNA fragments that hybridized with the probe was compared with that of fragments that hybridized with a nonsyntenic probe used as an internal control.

To evaluate the difference between the monosomic and disomic state, we compared normal men and women using a cloned X-specific DNA segment (DXS). The difference between men and women was in good correlation with the number of X chromosomes present and close to the 0.5 expected ratio: 0.54 ± 0.11. Similarly, the ratio *trisomy 11p/normal control* (1.35 ± 0.09) was compatible with the presence of a third β-globin gene locus in this patient. Since the β-globin gene cluster lies in the same region as the locus for lacticdehydrogenase A (LDHA), these results are in accordance with the sesquialteral gene dosage effect for LDHA previously demonstrated in this patient trisomic for 11p with the exception of band 11p13 [7]. These ratios of 0.54 for the monosomic state and 1.35 for the trisomic state indicate that this approach should be applicable to any cloned DNA segment that maps to a chromosome segment for which either a deletion or a duplication has been recognized.

We therefore used this method to measure the number of COLIA2 copies in a patient trisomic for the distal two-thirds of chromosome 7 that has been shown to carry the gene for COLIA2 [8]. The ratio *patient/normal control* (1.68 ± 0.28) was significantly increased and compatible with the presence of an extra copy of COLIA2. Therefore, the gene for COLIA2 can be assigned to the chromosomal segment that is duplicated in patient no. 2 (i.e., 7q21→7qter).

The existence of restriction fragment length polymorphism (RFLP) due to either qualitative or quantitative changes in DNA sequences may also provide a

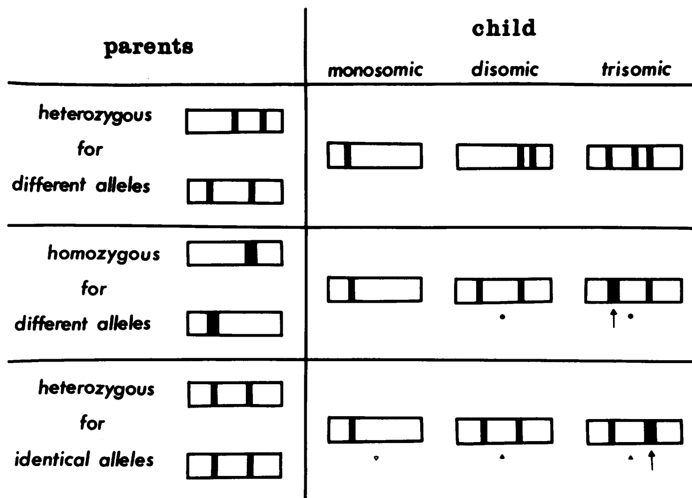


FIG. 4.—Direct gene dosage using an RFLP-linked probe. ▲ This profile must be compared, by densitometry, with the corresponding profiles of the parents. ● This profile should be compared with the profile of a mixture of equal amounts of DNA from both parents or with the profile from a heterozygous individual, belonging to the same family in order to decide whether the child is disomic or trisomic. ▽ This profile requires the use of a nonsyntenic probe as an internal control.

new tool for the regional assignment of DNA sequences by deletion mapping or trisomy mapping in aneusomic states [15]. It should be possible, in some cases, to establish whether a child is monosomic, disomic, or trisomic for a locus just by looking at the number and size of the DNA fragments that hybridize with a probe linked to a highly polymorphic locus [16] (fig. 4).

Conversely, densitometer tracing of the autoradiograms might prove to be a useful complementary test when parents are either heterozygous for two identical alleles or homozygous for two identical alleles, or homozygous for different alleles (fig. 4). This method should also permit the identification of the origin of the supernumerary or missing chromosome.

However, the detection of RFLP may require experiments with numerous different enzymes. The use of a nonsyntenic probe as an internal control may overcome difficulties inherent in the absence of any known linked RFLP. This method should be applicable to noncoding sequences as well as to coding sequences that may not be expressed in every tissue, or to genes coding for heteropolymeric proteins or enzymes for which specific techniques are lacking, provided the corresponding DNA sequences are available.

The method described here represents a new approach to prenatal and premorbid diagnosis of unbalanced chromosomal aberrations. It might be a useful complementary test or even a more precise and simpler diagnostic tool than cytogenetic analysis.

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