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Chromosomal Localization of the Human α_1-Antitrypsin Gene (PI) to 14q31-32

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SUMMARY

In situ hybridization of a recombinant cDNA probe containing the human α_1-antitrypsin gene to metaphase chromosomes demonstrated significant hybridization to chromosomal segment 14q31-32. A high percentage of cells analyzed (31%) displayed labeling on chromosome 14. Of all labeled sites on chromosome 14, 60% were found on segment 14q31-32. These results refine the previous assignment of the human α_1-antitrypsin gene to segment 14q24.1-32.1.

INTRODUCTION

α_1-Antitrypsin is a major protease inhibitor in human serum synthesized in the liver. The major physiological role of this protein is to protect the lungs from destruction by polymorphonuclear leukocyte elastase [1]. An imbalance between elastase and α_1-antitrypsin due to an inborn deficiency of α_1-antitrypsin may lead to the development of chronic obstructive pulmonary emphysema [2, 3]. In addition, α_1-antitrypsin deficiency may also be manifested in some affected individuals by the development of infantile liver cirrhosis [4].

Previous studies utilizing somatic cell hybrids [5] or genetic linkage analysis among family members [6] have localized the α_1-antitrypsin gene to chromosome segment 14q24.1-32.1. In this study, a recombinant plasmid containing a 1.43-kilobase (kb) cDNA insert specific for the human α_1-antitrypsin gene [7]

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was ^{3}H-labeled and hybridized directly to human metaphase chromosome preparations. Significant labeling of segment 14q31-32 was observed, allowing for finer mapping of the human α_1-antitrypsin gene to this chromosomal segment.

MATERIALS AND METHODS

Preparation of Plasmid DNA

The recombinant plasmid pAT83, containing a 1.43-kb human α_1-antitrypsin cDNA sequence inserted in the *Pst*I site of pBR322, was constructed as described [7]. Plasmid DNA was isolated from transformed *Escherichia coli* strain RRI by standard procedures [8].

Radiolabeling of DNA

pAT83 was ^{3}H-labeled by nick-translation using [^{3}H]dATP (51.1 Ci/mmol) and [^{3}H]dTTP (87.5 Ci/mmol) (New England Nuclear, Boston, Mass.) to a specific activity of 1.87×10^7 cpm/μg according to described procedures [9]. The reaction mixture was then fractionated on a Sephadex G-75 column. Labeled plasmid DNA was denatured and fractionated by electrophoresis in a 1% agarose gel to determine the size of single-stranded DNA fragments. The majority of single-stranded DNA lengths were found to be greater than 0.5 kb (data not shown). Single-strand fragments larger than 0.5 kb have been shown to result in higher efficiency of specific hybridization [10].

Preparation of Human Mitotic Chromosomes

Human metaphase chromosomes were prepared from whole blood cultures by standard techniques [11]. Chromosome preparations were stored for 1 week at room temperature and subsequently used within 14 days for in situ hybridization experiments.

In Situ Hybridization

In situ hybridization was performed by the method of Harper and Saunders [12]. Chromosome preparations were treated with pancreatic ribonuclease A (Sigma, St. Louis, Mo.) and denatured in 70% formamide (Eastman, Rochester, N.Y.) in 2 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M Na_3 citrate), pH 7.0., at 70°C for 2 min. Chromosomes were hybridized with ^{3}H-labeled pAT83 at 33–100 ng/ml in 50% formamide, 2 × SSC, 10% dextran sulfate (Pharmacia, Piscataway, N.J.), pH 7.0, for 12 hrs at 37°C. Following hybridization, slides were thoroughly rinsed in 50% formamide, 2 × SSC at 39°C, exposed to Kodak NTB2 nuclear track emulsion for 6–11 days at −80°C, and developed with Kodak Dektol at 15°C. Chromosomes were G-banded with 0.25% Wright stain for analysis [11].

RESULTS

In situ hybridization of a human α_1-antitrypsin cDNA probe to human metaphase chromosomes demonstrated significant labeling of segment 14q31-32. Hybridization of ^{3}H-labeled probe at concentrations of 33–100 ng/ml resulted in an average of 2.4 labeled sites per cell consisting of single silver grains. A total of 65 cells were analyzed and scored for the position of each labeled site (fig. 1). The distribution of labeled sites was visualized by plotting the data on a histogram. The abscissa is composed of an idiogram representing all human chromosomes placed head-to-tail and divided into 65 equal segments. The number of labeled sites was plotted along the ordinate in the middle of each segment. As shown in figure 1, the distal portion of chromosome 14 was

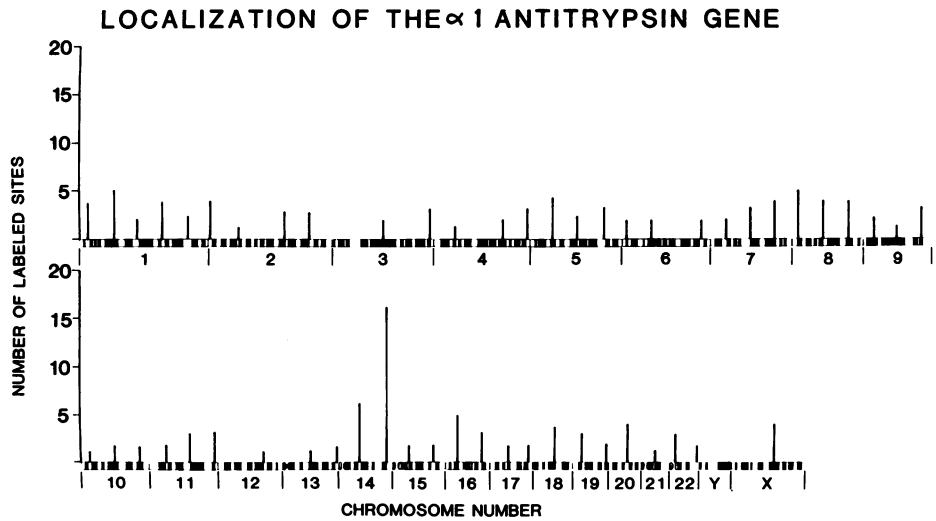

FIG. 1.—Distribution of 156 labeled sites from 65 hybridized cells illustrating significant labeling on the distal portion of the long arm of chromosome 14.

significantly labeled. Out of 65 cells, 31% (20/65) displayed labeling of chromosome 14. Distribution of all labeled sites on chromosomes 14 is displayed in figure 2. Chromosomal segment 14q31-32 contained 60% of labeled sites on chromosome 14 and 8% of all labeled sites. Figure 3 shows a typical cell with labeling of segment q31-32 on chromosome 14. Therefore, the human α_1-antitrypsin gene was assigned to chromosomal segment 14q31-32.

DISCUSSION

α_1-Antitrypsin plays an important role in the maintenance of good health. Deficiency of the α_1-antitrypsin protein predisposes individuals to infantile liver cirrhosis and chronic obstructive pulmonary emphysema [3, 4]. The human α_1-antitrypsin gene has previously been isolated [13] and shown to include three introns [14]. Sequencing of this gene demonstrated that α_1-antitrypsin deficien-

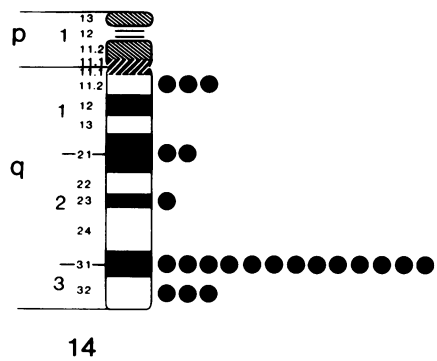

FIG. 2.—Idiogram of chromosome 14 displaying 60% of label on segment 14q31-32

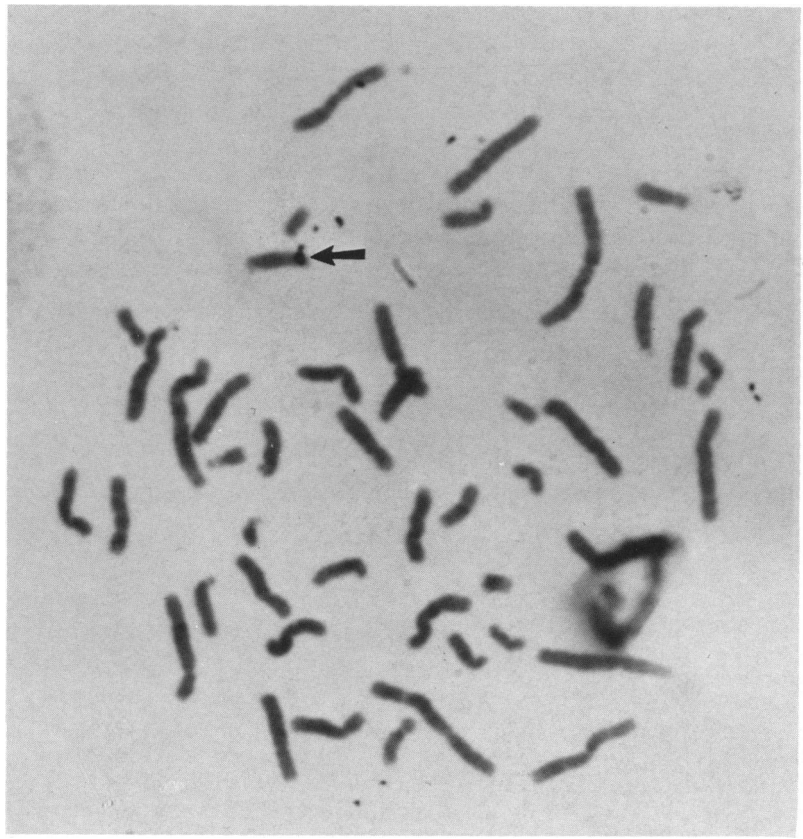

FIG. 3.—Representative metaphase cell hybridized with the ^{3}H-α_1-antitrypsin probe demonstrating typical labeling of 14q31-32.

cy was due to a single point mutation resulting in a substitution of lysine for glutamic acid at amino acid residue 342 in the protein [14].

Hybridization of the α_1-antitrypsin gene fragment to *Eco*RI-digested human genomic DNA has been shown to display hybridizing fragments of 9.6 kb and 8.5 kb [13]. Hybridization with a segment of DNA from one of the introns of the α_1-antitrypsin gene demonstrates that the authentic α_1-antitrypsin gene is located within the 9.6-kb band. Therefore, the 8.5-kb band contains a sequence that is closely related but not identical to the α_1-antitrypsin gene. Both sequences have been assigned to human chromosome 14 by Southern hybridization using DNA isolated from a panel of rodent/human cell hybrids [5].

Mapping of human genes is important in the study of disease and in understanding how groups of genes are regulated. Family linkage studies have localized the human α_1-antitrypsin gene to a large chromosomal segment 14q24.1-32.1 [6]. By annealing a ^{3}H-labeled cDNA probe to human metaphase chromosomes by in situ hybridization, we were able to further assign the α_1-antitrypsin gene to a smaller segment of the human genome, namely, 14q31-32. Since no other significantly labeled sites were observed, the data would suggest

that the α_1-antitrypsin-like gene present in an 8.5-kb *Eco*RI fragment may also be present in the chromosomal segment 14q31-32. Indeed, recent analysis of the human α_1-antitrypsin locus by cosmid cloning has demonstrated that the α_1-antitrypsin-related sequence is physically linked to the authentic gene (V. J. Kidd and S. L. C. Woo, manuscript in preparation).

Finally, using the 4.6-kb *Eco*RI fragment containing the 5′ end of the α_1-antitrypsin gene as a hybridization probe, extensive restriction fragment length polymorphism has been detected in the corresponding locus of the human genome [15]. Since the gene has been mapped to the 14q31-32 region, the α_1-antitrypsin gene can also serve as a valuable polymorphic marker for linkage analysis with other genetic loci in that region of human chromosome 14.

REFERENCES

1. LAURELL CB, JEPPSSON J-O: *The Plasma Proteins*, 2nd ed, edited by PUTMAN FW, New York, Academic Press, 1975, pp 229–264
2. OHLSSON K: Granulocyte collagenase and elastase and their interactions with α_1-antitrypsin and α_2-macroglobulin, in *Proteases and Biological Control*, edited by REICH E, RIFKIN D, SHAW E, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory, 1975, p 591
3. KUEPPERS F: *Lung Biology in Health and Disease*, vol. 2, edited by LITWIM S, New York, Marcel Dekker, 1978, pp 23–30
4. LAURELL CB, ERIKSSON S: The electrophoretic α_1-globulin pattern of serum in α_1-antitrypsin deficiency. *Scand J Clin Lab Invest* 14:490–494, 1963
5. LAI EC, KAO F, LAW ML, WOO SLC: Assignment of the α_1-antitrypsin gene and a sequence-related gene to human chromosome 14 by molecular hybridization. *Am J Hum Genet* 35:385–392, 1983
6. COX DW, MARKOVIC VD, TESHIMA IE; Gene for immunoglobulin heavy chains and for α_1-antitrypsin are localized to specific regions of chromosome 14q. *Nature* 297:428–430, 1982
7. LONG GL, CHANDRA T, WOO SLC, DAVIE EW, KURACHI K: Complete sequence of the cDNA for human α_1-antitrypsin and the gene for the S variant. *Biochemistry* 23:4828–4837, 1984
8. BOLIVAR F, RODRIGUEZ R, GRUNE PJ, ET AL.: Construction and characterization of new cloning vehicles. *Gene* 2:95–113, 1977
9. LAI EC, WOO SLC, DUGAICZYCK A, O'MALLEY BW: The ovalbumin gene: alleles created by mutations in the intervening sequences of the natural gene. *Cell* 16:201–221, 1979
10. MEINKOTH J, WAHL G: Hybridization of nucleic acids immobilized on solid supports. *Anal Biochem* 138:267–284, 1984
11. YUNIS JJ, CHANDLER ME: High-resolution chromosome analysis in clinical medicine, in *Progress in Clinical Pathology*, vol 7, edited by STEFANINI M, HOSSAINI AA, ISENBERG HD, New York, Grune & Stratton, 1977, pp 267–288
12. HARPER ME, SAUNDERS GF: Localization of single copy DNA sequences on G-banded chromosomes by *in situ* hybridization. *Chromosoma* 83:431–439, 1981
13. LEICHT M, LONG GL, CHANDRA T, ET AL.: Sequence homology and structural comparison between the chromosomal human α_1-antitrypsin and chicken ovalbumin genes. *Nature* 297:655–659, 1982
14. KIDD VJ, WALLACE RB, ITAKURA K, WOO SLC: α_1-Antitrypsin deficiency detection by direct analysis of the mutation in the gene. *Nature* 304:230–234, 1983
15. COX DW, MANSFIELD T, WOO SLC: A specific pattern of polymorphic DNA fragments occurs with α_1-antitrypsin deficiency allele, *PI Z*. *Am J Hum Genet* 36:134S, 1984