LOCALIZATION OF THE HUMAN PREALBUMIN GENE TO 18p11.1-q12.3 BY GENE DOSE EFFECT STUDY OF SOUTHERN BLOT HYBRIDIZATION

Yoshihiro JINNO,¹ Tadashi MATSUMOTO,¹ Tsutomu KAMEI,¹
Tatsuro KONDOH,^{1,2} Shuichiro MAEDA,³ Shukuro ARAKI,⁴
Kazunori SHIMADA,³ and Norio NIKAWA¹

¹*Department of Human Genetics and* ²*Department of Pediatrics,*
Nagasaki University School of Medicine, Sakamoto-machi,
Nagasaki 852, Japan

³*Department of Biochemistry and* ⁴*First Department of Internal*
Medicine, Kumamoto University Medical School, Honjo,
Kumamoto 860, Japan

Summary The human prealbumin gene was tried to map by the analysis of gene dose effects on Southern hybridization bands of various chromosome 18 abnormalities. The ³²P-labeled probe "pPA1" which contains the prealbumin cDNA hybridized to an 11 kb and a 4.6 kb *Hind* III-fragments from cells each of which has a different chromosome 18 constitution. Autoradiograms (hybridization bands) for pseudodicentric 18 (trisomy of almost whole of a chromosome 18) showed 1.5-fold density compared with those of the normal diploid. Autoradiograms for trisomy for 18q21.3-qter and monosomies for 18p11.2-pter and for 18q21.1-q21.3 were all not different in density from those of the diploid control. Our results provide evidence for regional assignment of this gene to 18p11.1-q12.3, most likely to cen-q12.3.

INTRODUCTION

Prealbumin (PA) is a plasma protein with a molecular weight of 54,980, which consists of four identical monomer subunits. This protein is involved in the transports of thyroxine and retinol-binding protein by binding them. Previous studies showed that a single amino acid substitution in PA is associated with a hereditary systemic amyloidosis, familial amyloidotic polyneuropathy (Dwulet and Benson, 1983; Tawara *et al.*, 1983; Nakazato *et al.*, 1984; Ueji *et al.*, 1984). The nucleotide sequence of cloned PA-cDNA has been identified (Mita *et al.*, 1984; Wallace

Received August 8, 1986; Accepted August 19, 1986

et al., 1985). Although the localization of the PA gene was assigned to chromosome 18 (Whitehead *et al.*, 1985; Wallace *et al.*, 1985), the regional locus of the gene has not yet been ascertained. This report deals with the regional mapping of the PA gene by the analysis of gene dose effects on Southern hybridization bands from various chromosome 18 abnormalities.

MATERIALS AND METHODS

Chromosome preparation. Five-hundred and fifty-band stage prometaphase chromosome preparations were made with the ethidium bromide technique (Ikeuchi and Sasaki, 1979) from three-day cultures of peripheral blood lymphocytes of three persons [MH, YH and a karyotypically normal person (NC)] and from cultures of four EBV-transformed lymphoblastoid cell lines (8011L, 7953L, 7307L and KY). The chromosomes were GTG-banded. Chromosome 18 constitutions of these seven samples are as follows: 8011L: 46,XX,inv(18) (p11.2q21.3); 7953L: 46,XY,rec(18),dup q inv(18)(p11.2q21.3)mat; 7307L: 46,XY,psu dic(18;18)(p11.32;p11.32); KY: mos46,XX/47,XX,+18 (5%/95%); MH: 46,XX,del(18)(p11.2p11.32); YH: 46,XX,del(18) (q21.1q21.3); NC: 46,XX.

Probe preparation. The prealbumin cDNA clone, pPA1, was isolated from a human liver cDNA library by screening with synthesized tetradecamer oligonucleotide probes (Mita *et al.*, 1984). The clone pPA1 consists of 669 nucleotides which contains 0.4 kb encoded information for 147 amino acids including whole human prealbumin sequence. The recombinant plasmid (Okayama-Berg vector) containing the prealbumin cDNA was ³²P-labeled by nick-translation and used as a probe. As probes for inner controls, a 0.65 kb of unique DNA sequence "AS5'e" and a 0.8 kb of unique sequence "2Ie" were also ³²P-labeled. AS5'e is a *PvuII/SmaI* fragment derived from the 5' flanking region of the argininosuccinate synthetase (ASS) gene, while 2Ie is a fragment obtained by *EcoRI*-digestion from the second intron of the ASS gene (Jinno *et al.*, 1985). The ASS gene is located on chromosome 9 (HGM8, 1985). The probes were hybridized to genomic DNA at a specific radioactivity of 8.5×10^5 – 1.0×10^6 cpm/lane.

DNA isolation and Southern blot hybridization. Nuclear DNA was extracted from peripheral blood leukocytes of MH, YH and NC and from the four lymphoblastoid cell lines. Each DNA sample (10 µg) was digested with a restriction endonuclease *HindIII* at 4 units per µg DNA at 37°C for 6 hr according to the condition specified by the supplier (Takara Shuzo Co., Ltd., Kyoto). DNA fragments were separated by electrophoresis in 0.8% agarose gel and transferred onto a nitrocellulose filter. Blotting, prehybridization, hybridization and washing were carried out according to a standard technique (Maniatis *et al.*, 1982). Autoradiography on Sakura X-ray films with an intensifying screen was performed at –70°C for 24–48 hr.

Densitometric analysis. The density of autoradiograms was analyzed compared

with that for inner controls by a densitometer in order to know a dosage effect of the PA gene in each chromosome 18 constitution.

RESULTS

The results of Southern blot hybridization of the samples from the seven cells each of which has a different chromosome 18 constitution were shown in Fig. 1. The probe pPA1 hybridized to an 11 kb and a 4.6 kb fragments in every sample examined. The density of these autoradiographic bands were well parallel with doses of DNA samples from a karyotypically normal person (Fig. 1). Autoradiograms of the hybridization with the probes AS5'e and 2Ie showed a 7.2 kb band and an 8.1 kb band (data not shown), respectively. Each band with the latter two probes were used as inner controls when analyzing the relative density of the PA bands. The PA band of the 7307L cell and that of the KY cell showed 1.51-fold and 1.34-fold density, respectively, compared with that of the NC cell (Fig. 2 and Table 1). On the other hand, autoradiograms of the cells 8011L, 7953L, MH and YH were all not different in density from those of the NC cells. These findings indicate that the locus of the PA gene is confined to the chromosome bands 18p11.1-q12.3, most likely to cen-q12.3.

Fig. 1. Southern hybridization bands of *Hind*III-digested DNA from the cell with various chromosome 18 constitutions, with a probe "pPA1" or simultaneously with two probes "pPA1" and "AS5'e." The numbers beside wedges show the length of a fragment of the prealbumin gene with kilo base pairs (kb), and asterisks denote a 7.2 kb fragment of the argininosuccinate synthetase gene as an inner control. The left three autoradiograms are those for 5 μ g, 7.5 μ g and 10 μ g of genomic DNA, respectively, from the normal diploid cell, showing a dose response density. The lane a, 8011L; b, 7953L; c, 7307L; d, KY; g, MH; h, YH; e and f, normal control cell.

Fig. 2. Gene doses of various chromosome 18 constitutions. The numbers in the lowest row indicate the copy number of the prealbumin gene. An ideogram in the right denotes the prealbumin gene (PA) locus.

Table 1. Densitometry of autoradiograms for various chromosome 18 abnormalities.

Probe	Density ratio	Relative density in cells examined						
		8011L	7953L	7307L	KY	MH	YH	NC
pPA1/AS5'e	(a)	0.96	0.93	1.29	1.14	0.80	—	0.87
	(b)	1.10	1.07	1.48	1.31	0.92	—	1.00
	(a)	1.10	1.16	1.88	1.61	—	—	1.24
	(b)	0.74	1.07	1.12	1.30	—	—	1.00
	(a)	—	—	—	—	—	0.59	0.63
	(b)	—	—	—	—	—	0.94	1.00
	(a)	—	—	—	—	—	0.54	0.55
	(b)	—	—	—	—	—	0.99	1.00
pPA1/2Ie	(a)	0.74	0.79	1.27	0.96	0.86	—	0.74
	(b)	1.00	1.07	1.71	1.30	1.16	—	1.00
	(a)	0.71	0.66	1.05	0.89	0.58	—	0.60
	(b)	1.18	1.10	1.75	1.48	0.97	—	1.00
	(a)	0.96	0.93	1.29	1.14	0.80	—	0.87
	(b)	1.10	1.07	1.48	1.31	0.92	—	1.00
	(a)	—	—	—	—	—	0.67	0.69
	(b)	—	—	—	—	—	0.97	1.00
	(a)	—	—	—	—	—	0.98	0.99
	(b)	—	—	—	—	—	0.99	1.00
	Mean (b)	1.02	1.08	1.51	1.34	0.99	0.97	1.00

(a): Density of prealbumin gene band/density of inner control band. (b): (a) for each cell/(a) for normal control.

DISCUSSION

Our results that the autoradiograms of the cell 7307L with pseudodicentric chromosome 18 (trisomy of almost whole of a chromosome 18) showed an 1.5-fold density, *i.e.* three copies of the PA gene, compared with those of the karyotypically normal control provide evidence for assignment of the gene to chromosome 18, supporting the previous studies (Whitehead *et al.*, 1984; Wallace *et al.*, 1985). The gene copy number between 2 and 3 in the cell line KY, which was estimated by densitometry, may reflect the mosaicism. The density showing two copies in the cell lines 7953L (trisomy for 18q21.3-qter), the cell MH (monosomy for 18p11.2-pter) and in the cell YH (monosomy for 18q21.1-q21.3) further indicates that the PA gene locus is confined to chromosome regions 18p11.1-q12.3, most likely to cen-q12.3. Since the locus of a gene should generally be assigned with at least two different ways of ascertainment, it would be necessary to confirm our conclusion by *in situ* hybridization.

An assignment of the PA gene to narrower region(s) will be performed by analyzing a structural chromosome abnormality involving the paracentromeric regions of chromosome 18. Another strategy for regional mapping of the gene may include a linkage study with other gene markers. However, a very few genes or markers have been known on chromosome 18 (HGM8, 1985), and only a possible gene to be used is the thymidylate synthetase gene of which presumed locus is in the range of 18pter-q12 (Hori *et al.*, 1985). Therefore, our conclusion of assignment may conversely help in the localization of other genes. In other words, the PA gene is an appropriate gene marker on chromosome 18.

Acknowledgments The lymphoblastoid cell lines 8011L, 7953L and 7307L were kindly made available by Dr. Tatsuro Ikeuchi, Department of Cytogenetics, Medical Research Institute, Tokyo Medical and Dental University, the cell line KY by Dr. Ichiro Murano, Department of Pediatrics, Yamaguchi University School of Medicine, and blood of YH by Dr. Ikuko Kondo, Department of Human Genetics, Institute of Basic Medical Sciences, University of Tsukuba. We also wish to thank Dr. Yasuhiko Kaneko, Department of Laboratory Medicine, Saitama Cancer Centre, and Dr. Yoshitsugu Sugio, Division of Medical Genetics, Kanagawa Children's Medical Center, for giving us several cell lines for a preliminary study. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (no. 60480468).

Note Added in Proof: After submitting this paper for publication, we found a study of *in situ* hybridization (Sparks *et al.*, 1986) in which the PA gene locus was assigned to almost the same region as our assignment.

REFERENCES

- Dwulet, F.E. and Benson, M.D. 1983. Polymorphism of human plasma thyroxin binding prealbumin. *Biochem. Biophys. Res. Commun.* **114**: 657-662.

- Human Gene Mapping 8, Helsinki Conference (1985), Eighth International Workshop on Human Gene Mapping. 1985. *Cytogenet. Cell Genet.* **40**: 250.
- Hori, T., Ayusawa, D., Shimizu, K., Koyama, H., and Seno, T. 1985. Assignment of human gene encoding thymidylate synthase to chromosome 18 using interspecific cell hybrids between thymidylate synthase-negative mouse mutant cells and human diploid fibroblasts. *Somat. Cell Mol. Genet.* **11**: 277-283.
- Ikeuchi, T. and Sasaki, M. 1979. Accumulation of early mitotic cells in ethidium bromide-treated human lymphocyte cultures. *Proc. Jpn. Acad.* **55**: 15-18.
- Jinno, Y., Matuo, S., Nomiya, H., Shimada, K., and Matsuda, I. 1985. Novel structure of the 5' end region of the human argininosuccinate synthetase gene. *J. Biochem.* **98**: 1395-1403.
- Maniatis, T., Fritsch, E.F., Sambrook, J. 1982. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp. 150-172, 382-389.
- Mita, S., Maeda, S., Shimada, K., and Araki, S. 1984. Cloning and sequence analysis of cDNA for human prealbumin. *Biochem. Biophys. Res. Commun.* **124**: 558-564.
- Nakazato, M., Kangawa, K., Minamino, N., Tawara, S., Matsuo, H., and Araki, S. 1984. Identification of a prealbumin variant in the serum of a Japanese patient with familial amyloidotic polyneuropathy. *Biochem. Biophys. Res. Commun.* **122**: 712-718.
- Sparkes, R.S., Sasaki, H., Mohandas, T., Yoshioka, K., Klisak, I., Sakaki, Y., Heinzmann, C., and Simon, M.I. 1986. Personal communication through K. Yoshioka.
- Tawara, S., Nakazato, M., Kangawa, K., Matsuo, H., and Araki, S. 1983. Identification of amyloid prealbumin variant in familial amyloidotic polyneuropathy (Japanese type). *Biochem. Biophys. Res. Commun.* **116**: 880-888.
- Ueji, M., Suzuki, T., Higa, S., Sakoda, S., Kishimoto, S., Titani, K., Takio, K., Hayashi, A., Takada, Y., and Nakajima, A. 1984. Genetic studies of familial amyloid polyneuropathy in the Arao district of Japan. III. Analysis of amyloid fibril protein. *Jpn. J. Human Genet.* **29**: 311-325.
- Wallace, M.R., Naylor, S.L., Kluge-Beckerman, B., Long, G.L., McDonald, L., Shows, T.B., and Benson, M.D. 1985. Localization of the human prealbumin gene to chromosome 18. *Biochem. Biophys. Res. Commun.* **129**: 753-758.
- Whitehead, A.S., Skinner, M., Bruns, G.A.P., Costello, W., Edge, M.D., Cohen, A.S., and Sipe, J.D. 1984. Cloning of human prealbumin complementary DNA. Localization of the gene to chromosome 18 and detection of a variant prealbumin allele in a family with familial amyloid polyneuropathy. *Mol. Biol. Med.* **2**: 411-423.