Volume 247, number 1, 123–126 FEB 07015 April 1989

The prostate-specific antigen gene and the human glandular kallikrein-1 gene are tandemly located on chromosome 19

P.H.J. Riegman, R.J. Vlietstra, P. Klaassen, J.A.G.M. van der Korput, A. Geurts van Kessel[+], J.C. Romijn* and J. Trapman

Departments of Pathology, [+]Cell Biology and Genetics and **Urology, Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands*

Received 16 February 1989

Using a prostate-specific antigen cDNA as a hybridization probe, clones containing the kallikrein genes encoding prostate-specific antigen, human glandular kallikrein-1 and pancreas/kidney kallikrein were isolated from a human genomic library. Clones containing the prostate-specific antigen gene and the human glandular kallikrein-1 gene overlap and span a region of about 36 kb. The two genes are aligned in a head to tail orientation at a mutual distance of 12 kb. Southern blot analysis of DNA from a panel of human-hamster hybrid cells with specific probes revealed the genes to be situated on chromosome 19. Assuming that the pancreas/kidney kallikrein gene is located in the same cluster, the distance to the prostate-specific antigen gene and the human glandular kallikrein gene must be at least 15 kb.

Kallikrein; Chromosome 19; Prostate-specific antigen; Glandular kallikrein; (Human)

1. INTRODUCTION

Kallikreins and kallikrein-like proteins are a subgroup of the serine protease family which shows a high degree of substrate specificity (for recent reviews see [1,2]). In mouse and rat kallikreins are encoded by a large multigene family [3–7]. In the mouse genome at least 24 kallikrein genes have been identified, which are clustered in groups of up to 11 genes on chromosome 7 [3]. Twelve of the mouse kallikrein genes appear to be pseudogenes [6]. A family of 15 to 20 kallikrein genes has been found in the rat genome [5]. At least 4 of these genes are functional [4]. Three human kallikrein genes have been described: prostate-specific antigen (PA) [8–10], human glandular kallikrein (hGK-1) [11] and (pancreas/kidney) kallikrein (hPK) [12–14]. Both the PA and hGK-1 gene are completely sequenced, their mutual homology is 82% [10,11]. PA is exclusively synthesized by the epithelial cells of the prostate gland [15–18]. Its presumed function is dissolving the seminal coagulum by digesting proteins secreted by the seminal vesicles, that cause the gel-like structure of the semen [19,20]. An elevated level of PA in the serum is a reliable marker for prostate cancer [21–24]. Expression of the hGK-1 gene, like that of PA, seems also to be restricted to the prostate [25]. The physiological function of hGK-1 has not yet been established. hPK is a protease that cleaves the precursor kininogen to release small vasoactive peptides or kinins [26]. It has been suggested that the kallikrein-kinin system is involved in hypertension [27].

Recently, the hPK gene has been mapped on chromosome 19 [14]. In this study the chromosomal location of the PA and hGK-1 genes is described and the size and presumed clustering of the human kallikrein gene family are discussed.

Correspondence address: P.H.J. Riegman, Department of Pathology, Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands

2. MATERIALS AND METHODS

A human genomic library (partially *Mbo*I-digested DNA in lambda EMBL 3) was kindly provided by Dr G. Grosveld (Rot-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

terdam). The library was screened with a ^{32}P-labeled [28] 380 bp *Eco*RI-*Sac*I fragment derived from the PA cDNA clone PA 525 [9]. Duplicate nitrocellulose filters were hybridized overnight at 65°C in 6 × SSC containing 10 × Denhardt, 0.1% SDS, and 100 μg/ml salmon sperm DNA. Filters were washed twice in 3 × SSC for 20 min at 65°C, twice in 1 × SSC for 20 min at 65°C and once in 0.3 × SSC for 10 min at 65°C. Filters were exposed to X-ray films for 18 h at −70°C using intensifying screens. Positive phages were purified by two isolation cycles. Phages were propagated, DNA isolated and a restriction map was made using standard procedures [29]. Fragments were subcloned in a plasmid vector for detailed mapping. For sequencing fragments were subcloned in M13mp18/19. Sequencing was done by the dideoxy chain termination method [30], using sequenase (USB, Cleveland).

Somatic cell hybrids were generated by fusion of human cells with the hamster A3 cells line as described [31]. DNA of 15 of these hybrid cell lines was isolated and analyzed by Southern blotting using standard procedures. The probe used for hybridization was the 1600 bp cDNA clone PA 525 [9]. The labeling, hybridization and washing conditions were identical to those used for the screening of the genomic library as described above.

3. RESULTS AND DISCUSSION

A fragment of a PA cDNA clone was used to screen a genomic library (see section 2). Four different clones (designated 4P1 (18.5 kb), 5P1 (15.5 kb), 7P1 (17 kb) and 9P1 (18 kb)) were isolated. Partial restriction maps of 4P1, 5P1 and 9P1 are shown in fig.1A, that of 7P1 is depicted in fig.1B. Sequence analysis of specific fragments proved that 7P1 contains the hPK gene [14] and 9P1 the hGK-1 gene [11]; 4P1 and 5P1 largely overlap and both contain the complete PA gene. The exon/intron organization and the sequence of the PA gene has been described in detail elsewhere [10]. The organization of the hGK-1 and hPK gene is derived from [11] and [14]. Hybridization experiments with terminal fragments revealed that 4P1/5P1 and 9P1 contain a small common region. In agreement with this finding, a 3′-fragment of 4P1/5P1 and a 5′-fragment of 9P1 hybridized with the same band in *Bam*HI, *Hin*dIII and *Bam*HI + *Hin*dIII digests of genomic DNA (data not shown). Comparison of the restriction maps of 4P1/5P1 and 9P1 with that of the earlier described clone λHGK1 [11] confirmed the tandem arrangement on the PA and hGK-1 gene. The two genes are positioned in a head to tail orientation and are separated by a stretch of approximately 12 kb. Together the clones span a region of 36 kb.

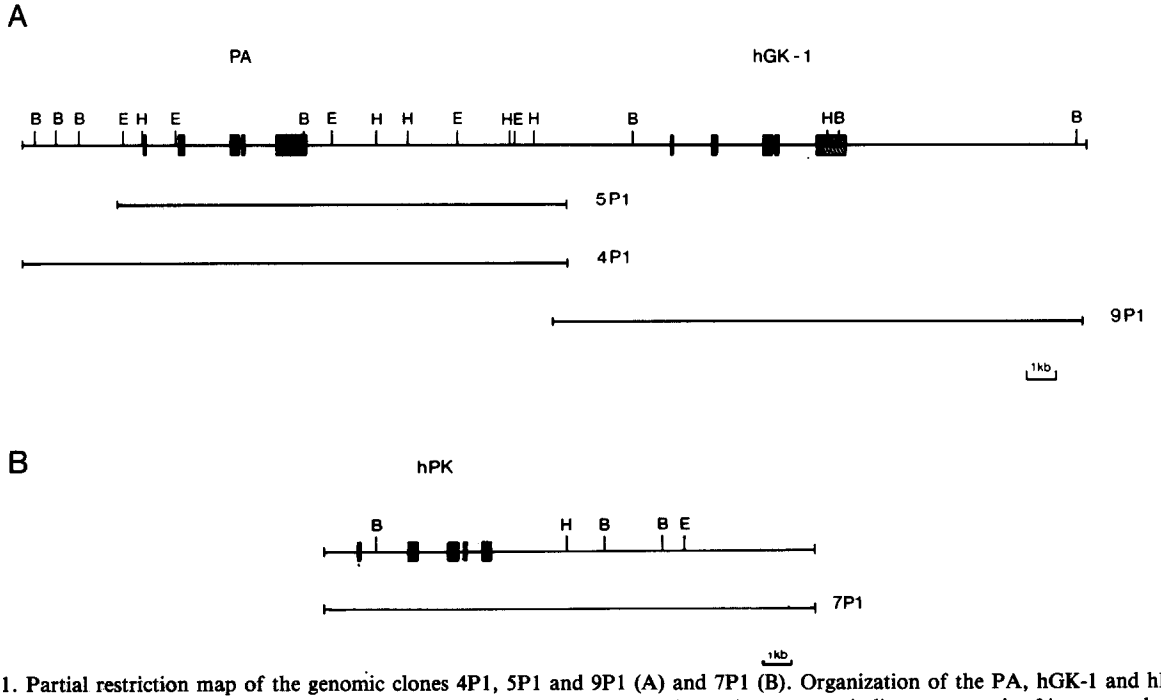

Fig.1. Partial restriction map of the genomic clones 4P1, 5P1 and 9P1 (A) and 7P1 (B). Organization of the PA, hGK-1 and hPK gene is from [10,11,14]. The closed boxes represent exons. The hatched area in the hGK-1 gene indicates a putative 3′-nontranslated region of the cDNA. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII.

In the mouse genome a large kallikrein gene family (about 25 genes) is found to be located in clusters on chromosome 7 [6]. Similarly, the rat kallikrein gene family counts 15–20 members [5]. Southern blot analysis of genomic DNA with hGK-1 and hPK probes indicated that the human kallikrein family is much smaller, and it has been suggested that it comprises only three genes [11,13]. To substantiate these observations, similar experiments were carried out using a PA cDNA probe (fig.2). In all digests only a limited number of bands could be visualized. Because the complete sequence of the PA gene [10], the hGK-1 gene [11] and most of the hPK gene has been published [14] and restriction maps of the corresponding genomic areas are available it could be deduced that the hybridizing genomic fragments represent the complete PA, hGK-1 and hPK genes or parts of the three genes. Under the same conditions the PA probe detected a large series of kallikrein genes in mouse DNA (data not shown). These findings firmly established that the human kallikrein gene family indeed encompasses only three closely related genes: PA, hGK-1 and hPK.

To determine the chromosomal location of the PA and hGK-1 gene, *Bam*HI-digested DNA from 15 different human-hamster cell hybrids containing characteristic sets of human chromosomes was analyzed by Southern blotting using a PA cDNA probe. Examples of the experiments are shown in fig.3. In control human DNA three bands are detected (fig.3B). Two of these bands (a weak 13 kb signal and a strong 8 kb signal) represent the PA gene; the 7.6 kb fragment contains the hGK-1 gene; the predicted 8 kb hPK fragment cannot be seen in this experiment. Hamster DNA does not cross-hybridize under the conditions used (data not shown). The results, as summarized in table 1, clearly show that the PA and the hGK-1 gene are situated on chromosome 19. Recently, the hPK gene was reported to be located on the same chromosome [14]. Clustering of the human kallikrein genes would be in accordance with the close linkage of kallikrein genes found in the mouse genome [6]. In this species the distance between the genes in the various clusters can be as small as 3–5 kb. It was already concluded that the region between the PA gene and the hGK-1 gene is considerably larger (about 12 kb, see fig.1A). It seems reasonable to assume that, if the kallikrein

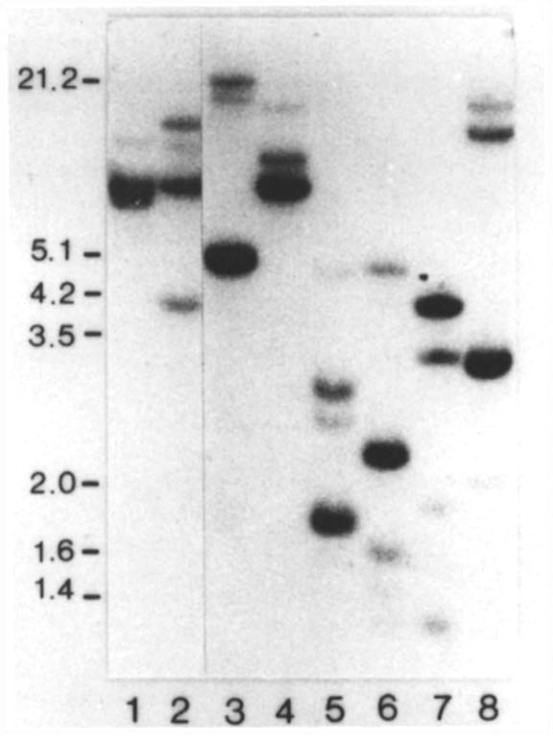

Fig.2. Southern blot analysis of human genomic DNA hybridized with PA cDNA clone 525 (see [10]). DNA was digested with *Bam*HI (lane 1); *Bgl*II (2); *Eco*RI (3); *Hin*dIII (4); *Nco*I (5); *Pst*I (6); *Pvu*II (7); *Sac*I (8).

family is clustered, the hPK gene is aligned in the same orientation as the PA and hGK-1 gene. In this case a minimal distance of 15 kb between hPK and PA or hGK-1 can be predicted from the restriction maps of the clones presented in fig.1 combined with those of two other published clones [11,14]. Alternatively, although located on the same chromosome, the hPK gene may not be close-

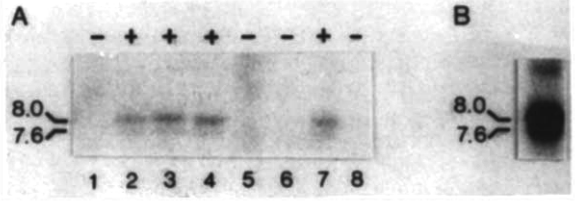

Fig.3. Southern blot analysis of DNA from human-hamster hybrid cells (lanes 1–8) (A) and human control DNA (B) hybridized with PA 525 (see [10]). DNA was digested with *Bam*HI. The 8.0 kb band represents the PA gene, the 7.6 kb band the hGK-1 gene. A positive hybridization signal is indicated by a +.

Table 1

Correlation between the presence or absence of PA and hGK-1 and human chromosomes in the human-hamster hybrid cell lines

Chromosome	Chromosome/PA + hGK-1 (no. of hybrids)				Concordance (%)
	+/+	−/−	+/−	−/+	
1	5	2	3	5	47
2	2	5	0	8	47
3	4	3	2	6	47
4	8	2	3	2	67
5	6	2	3	4	53
6	7	2	3	3	60
7	6	2	3	4	53
8	6	4	1	4	67
9	5	2	3	5	47
10	4	3	2	6	47
11	7	0	5	3	47
12	8	1	4	2	60
13	4	3	2	6	47
14	6	2	3	4	53
15	6	3	2	4	60
16	8	3	2	2	73
17	10	0	5	0	67
18	5	3	2	5	53
19	10	5	0	0	100
20	8	2	3	2	67
21	7	1	4	3	53
22	8	2	3	2	67
X	5	2	3	5	47
Y	0	4	1	10	26

ly linked to PA and hGK-1. Additional chromosome walking or pulse-field electrophoresis experiments will have to settle this point.

Acknowledgements: We are indebted to Mrs Paula Delfos for photography. This work has been supported by a grant from the Netherlands Cancer Foundation (KWF).

REFERENCES

[1] Drinkwater, C.C., Evans, B.A. and Richards, R.I. (1988) Trends Biochem. Sci. 13, 169–172.
[2] Macdonald, R.J., Margolius, H.S. and Erdös, E.G. (1988) Biochem. J. 253, 313–321.
[3] Mason, A.J., Evans, B.A., Cox, D.R., Shine, J. and Richards, R.I. (1983) Nature 303, 300–307.
[4] Ashley, P.L. and MacDonald, R.J. (1985) Biochemistry 24, 4512–4520.
[5] Gerald, W.L., Chao, J. and Chao, L. (1986) Biochim. Biophys. Acta 866, 1–14.
[6] Evans, B.A., Drinkwater, C.C. and Richards, R.I. (1987) J. Biol. Chem. 262, 8027–8034.
[7] Chen, Y.P., Chao, J. and Chao, L. (1988) Biochemistry 27, 7189–7196.
[8] Lundwall, A. and Lilja, H. (1987) FEBS Lett. 214, 317–322.
[9] Riegman, P.H.J., Klaassen, P., Van der Korput, J.A.G.M., Romijn, J.C. and Trapman, J. (1988) Biochem. Biophys. Res. Commun. 155, 181–188.
[10] Riegman, P.H.J., Vlietstra, R.J., Van der Korput, J.A.G.M., Romijn, J.C. and Trapman, J. (1989) Biochem. Biophys. Res. Commun., in press.
[11] Schedlich, L.J., Bennetts, B.H. and Morris, B.J. (1987) DNA 6, 429–437.
[12] Fukushima, D., Kitamura, N. and Nakanishi, S. (1985) Biochemistry 24, 8037–8043.
[13] Baker, A.R. and Shine, J. (1985) DNA 4, 445–450.
[14] Evans, B.A., Yun, Z.X., Close, J.A., Tregear, G.W., Kitamura, N., Nakanishi, S., Callen, D.F., Baker, E., Hyland, V.J., Sutherland, G.R. and Richards, R.I. (1988) Biochemistry 27, 3124–3129.
[15] Wang, M.C., Valenzuela, L.A., Murphy, G.P. and Chu, T.M. (1979) Invest. Urol. 17, 159–163.
[16] Wang, M.C., Papsidero, L.D., Kuriyama, M., Valenzuela, L.A., Murphy, G.P. and Chu, T.M. (1981) The Prostate 2, 89–96.
[17] Watt, K.W.K., Lee, P.J., M'Timkulu, T., Chan, W.P. and Loor, R. (1986) Proc. Natl. Acad. Sci. USA 83, 3166–3170.
[18] Gallee, M.P.W., Van Vroonhoven, C.C.J., Van der Korput, J.A.G.M., Van der Kwast, T.H., Ten Kate, F.J.W., Romijn, J.C. and Trapman, J. (1986) The Prostate 9, 33–45.
[19] Lilja, H. (1985) J. Clin. Invest. 76, 1899–1903.
[20] McGee, R.S. and Herr, J.C. (1988) Biol. Reprod. 39, 499–510.
[21] Kuriyama, M., Wang, M.C., Papsidero, L.D., Kilian, C.S., Shimano, T., Nishiura, T., Valenzuela, L., Murphy, G.P. and Chu, T.M. (1980) Cancer Res. 40, 4658–4662.
[22] Killian, C.S., Yang, N., Emrich, L.J., Vargas, F.P., Kuriyama, M., Wang, M.C., Slack, N.H., Papsidero, L.D., Murphy, G.P. and Chu, T.M. (1985) Cancer Res. 45, 886–891.
[23] Killian, C.S., Emrich, L.J., Vargas, F.P., Yang, N., Wang, M.C., Priore, R.L., Murphy, G.P. and Chu, T.M. (1986) J. Natl. Cancer Inst. 76, 179–185.
[24] Stamey, T.A., Yang, N., Hay, A.R., McNeal, J.E., Freiha, F.S. and Redwine, E. (1987) New Engl. J. Med. 317, 909–916.
[25] Chapdelaine, P., Paradis, G., Tremblay, R.R. and Dube, J.Y. (1988) FEBS Lett. 236, 205–208.
[26] Schachter, M. (1980) Pharmacol. Rev. 31, 1–17.
[27] Carretero, O.A. and Scilci, A.G. (1980) Am. J. Physiol. 238, F247–F255.
[28] Feinberg, A.P. and Vogelstein, P. (1983) Anal. Biochem. 132, 6–13.
[29] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[30] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[31] Geurts van Kessel, A., Tetteroo, P.A.T., Von dem Borne, K., Hagemeijer, A. and Bootsma, D. (1983) Proc. Natl. Acad. Sci. USA 80, 3748–3752.