Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 8548–8552, December 1987
Genetics

Plasminogen activator inhibitor type 1 gene is located at region q21.3-q22 of chromosome 7 and genetically linked with cystic fibrosis

(protease inhibitor/serpin/gene mapping)

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Communicated by Lester O. Krampitz, August 13, 1987 (received for review February 19, 1987)

ABSTRACT **The regional chromosomal location of the human gene for plasminogen activator inhibitor type 1 (*PAI1*) was determined by three independent methods of gene mapping. *PAI1* was localized first to 7cen-q32 and then to 7q21.3-q22 by Southern blot hybridization analysis of a panel of human and mouse somatic cell hybrids with a PAI1 cDNA probe and *in situ* hybridization, respectively. We identified a frequent *Hin*dIII restriction fragment length polymorphism (RFLP) of the *PAI1* gene with an information content of 0.369. In family studies using this polymorphism, genetic linkage was found between *PAI1* and the loci for erythropoietin (*EPO*), paraoxonase (*PON*), the *met* protooncogene (*MET*), and cystic fibrosis (*CF*), all previously assigned to the middle part of the long arm of chromosome 7. The linkage with *EPO* was closest with an estimated genetic distance of 3 centimorgans, whereas that to *CF* was 20 centimorgans. A three-point genetic linkage analysis and data from previous studies showed that the most likely order of these loci is *EPO, PAI1, PON, (MET, CF)*, with *PAI1* being located centromeric to *CF*. The *PAI1* RFLP may prove to be valuable in ordering genetic markers in the CF-linkage group and may also be valuable in genetic analysis of plasminogen activation-related diseases, such as certain thromboembolic disorders and cancer.**

Proteolysis caused by plasminogen activation is involved in many diverse biological processes (1–5). The two types of mammalian plasminogen activators, urokinase-type (u-PA) and tissue-type (t-PA), are both serine proteases with similar catalytic specificities but are products of different genes, located on chromosomes 10 and 8, respectively (6–10).

Plasminogen activation is regulated at several levels—e.g., by hormonal regulation of the synthesis of the activators and by modulators of their enzyme activity. One such modulator is plasminogen activator inhibitor type 1 (PAI1), an ≈50-kDa glycoprotein produced by endothelial cells and several neoplastic cell lines and found in thrombocytes and blood plasma (11–16). PAI1 inhibits u-PA and t-PA and belongs to the group of serpins, being an argserpin with an arginine residue at the reactive center (17–20).

We have previously described the derivation of monoclonal antibodies to PAI1 and their use for purification of the inhibitor (21). These antibodies and amino acid sequence data have allowed the identification of PAI1 cDNA clones from two different sources (17, 18). The *PAI1* gene was assigned to human chromosome 7 by hybridization analysis of chromosomes sorted onto filters (18).

In the present study the results of cell hybrid and *in situ* hybridization analysis placed *PAI1* in the area 7q21-q22, close to the chromosomal location predicted for cystic fibrosis (CF) on the basis of genetic linkage between CF and a number of polymorphic loci (22–28). CF is a common autosomal recessive disease with a carrier frequency of 1:20 in Caucasian populations. The basic biochemical defect in CF is as yet unknown and further genetic linkage studies are needed to identify the responsible genetic locus. We therefore identified a restriction fragment length polymorphism (RFLP) for *PAI1* and analyzed genetic linkage between the *PAI1* gene and CF as well as other genes located in this region.

MATERIALS AND METHODS

DNA Probes. The construction and characterization of the PAI1 cDNA clone have been described elsewhere (17). A 2.0-kilobase (kb) *Bam*HI insert of pPAI1-A1 plasmid containing the entire coding sequence for PAI1 was used for polymorphism screening and linkage studies. ^{32}P-labeled probes were synthesized by using the procedure of Feinberg and Vogelstein (29, 30).

Other DNA polymorphisms used for linkage analysis have been described already: metD and metH (24, 25), Epo (28). The markers at the *MET* locus were analyzed as a haplotype, assuming no recombination between the *Taq* I sites and linkage equilibrium (24).

Somatic Cell Hybrids. The somatic cell hybrids used in this study were produced by the fusion of a mouse cell line (RAG or A9) with human cells, including cells containing translocation derivatives of chromosome 7. Determination of the chromosomal complement of the hybrids was performed by analysis of G-banded chromosomes combined with testing of known biochemical markers on chromosome 7 (31), essentially as reported (32). Human GM 3098 and A431 and mouse RAG cell lines were used as controls.

DNA Analysis. DNA from human, mouse, and mouse–human cells was prepared using standard techniques. Gene mapping using DNA panels of hybrid cell lines and RFLP analyses were carried out by using a modified form of

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Abbreviations: u-PA, urokinase-type plasminogen activator; t-PA, tissue-type plasminogen activator; PAI1, plasminogen activator inhibitor type 1; RFLP, restriction fragment length polymorphism; CF, cystic fibrosis; PON, paraoxonase; lod, logarithm of odds; EPO, erythropoietin; cM, centimorgans.

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Southern blotting (33) and nylon membranes [Genetrans (Plasco) or Hybond-N (Amersham)]. The membranes were hybridized to ^{32}P-labeled pPAI1-A1 DNA. After hybridization the filters were washed in successive changes of decreasing concentrations of NaCl/sodium citrate to a final wash in 15 mM NaCl/1.5 mM sodium citrate/0.1% $NaDodSO_4$ at 65°C. Autoradiography was at −70°C.

Paraoxonase (PON) Analysis. Six families were tested for the serum arylesterase PON, as described (34).

Chromosomal *in Situ* Hybridization. Hybridization *in situ* was performed on metaphase chromosomes essentially as described by Harper and Saunders (35).

Pedigrees and Families. Probands in inbred populations were identified, and the pedigrees were constructed as described (36, 37). Two large extended kindreds with CF were studied, one Amish Mennonite family containing 21 affected individuals and a Hutterite family containing 9 affected individuals. Seventeen two-generation nuclear families each with two (11 families) or three (6 families) affected children were also used in the linkage analysis. The establishment in these families of linkage between the loci and markers *COLIA2*, *EPO* (erythropoietin), *PON*, *D7S8*, *MET*, and *CF* has been reported (28, 37, 38).

Linkage Analysis. Two-point analyses and multipoint analyses were performed using the MLINK and LINKMAP programs, respectively, of the computer package LINKAGE (39). Logarithm of odds (lod) scores z were calculated by assuming no sex difference in recombination. Maximal likelihood estimates of the lod score ($\hat{z}$) and recombination fraction ($\hat{\theta}$) were calculated by using the computer program VACO3 (40). The confidence interval for the maximal likelihood estimate of the recombination fraction was determined by graphic interpolation (41). The large Amish Mennonite pedigree was divided into three sections to include one inbreeding loop in each section, thus simplifying the analysis and preserving to a large extent the complex interrelationships and common ancestry of the members of this pedigree.

RESULTS

Chromosomal Localization of the *PAI1* Gene. The chromosomal localization of the human gene for *PAI1* was identified by DNA hybridization in mouse–human hybrid cell lines containing different human chromosomes. Hybridization with the PAI1 cDNA probe to Southern blots of *Hin*dIII-digested DNA revealed two DNA fragments of 22 kilobase pairs (kbp) and 18 kbp (see below and Fig. 2). The presence of one or both of these fragments segregated in a variety of cell lines specifically with the human chromosome 7, whereas discordance was found with all other human chromosomes (data not shown). To study the sublocalization of the *PAI1* gene, 7 cell lines that contained specific regions of human chromosome 7 (Fig. 1) were investigated. As it appears from Fig. 2, a positive signal was only observed in those cell lines

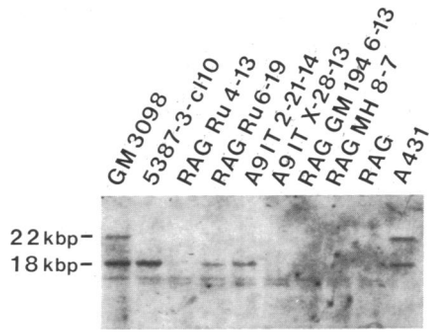

FIG. 2. Sublocalization of the human *PAI1* gene on chromosome 7 by using mouse–human somatic cell hybrids. Human (GM 3098, A431), mouse (RAG, A9), and hybrid (as indicated in Fig. 1) cell DNA was cleaved with *Hin*dIII restriction endonuclease, resolved by electrophoresis, and blotted onto membranes hybridized with ^{32}P-labeled plasmid containing a 2.0-kbp insert of human PAI1 cDNA, and specific hybridization was detected by autoradiography. From the autoradiogram it can be seen that the probe detects fragments of 22 kbp and/or 18 kbp in the human GM 3098 and A431 cells and in the hybrids 5387-3-cl10, RAG Ru 6-19, and A9 IT 2-21-14. A faint hybridization signal of 18 kbp was also detected for hybrid RAG GM 194 6-13 on a longer exposure. This reflects that *PAI1* was present in ≈30% of the cells in this hybrid line. As shown in Fig. 1, the only human chromosomal region these cells have in common is that located between the centromere and band 32 of the long arm of chromosome 7 (7cen-q32).

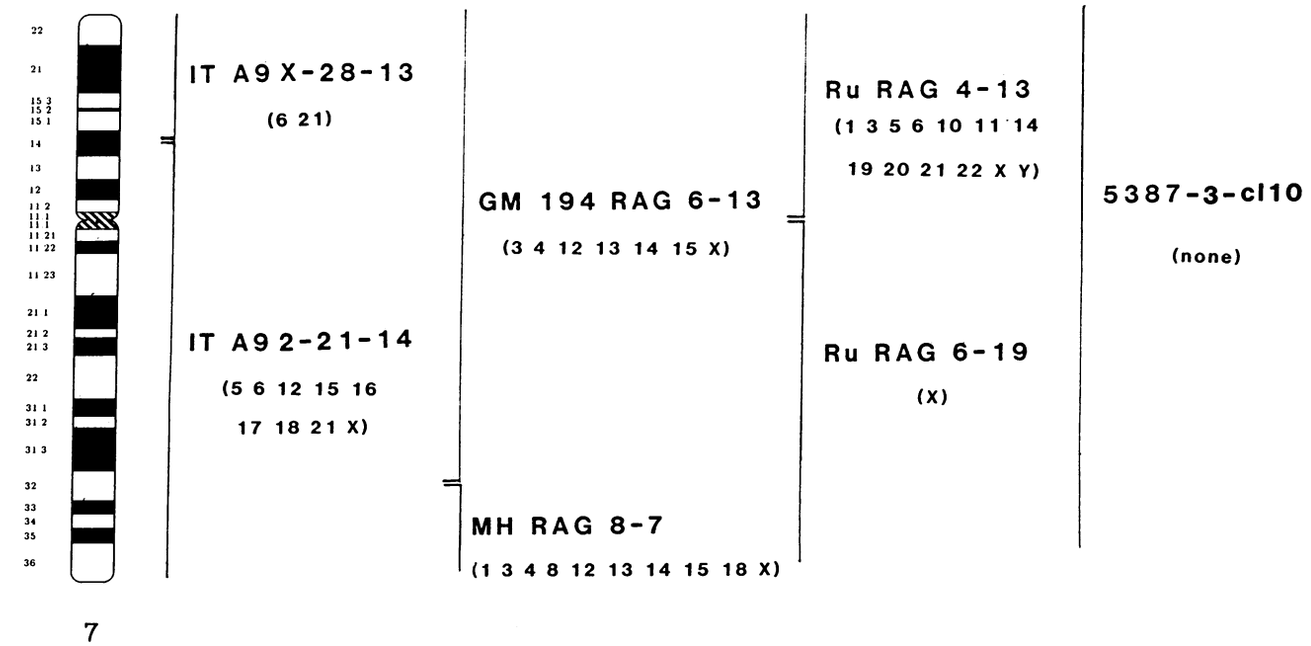

FIG. 1. Portion of human chromosome 7 retained in mouse–human hybrid cell lines used for sublocalization of the *PAI1* gene by Southern blotting analysis. In parentheses are indicated other human chromosomes (or derivatives) present in the respective cell lines. As illustrated in Fig. 2, *PAI1* is found in cell lines IT A9 2-21-14, GM 194 RAG 6-13, Ru RAG 6-19, and 5387-3-cl10.

that had retained the region of the long arm of chromosome 7 located between the centromere and band 32 (7cen-q32).

As an alternative approach, the localization of the *PAI1* gene was studied by *in situ* hybridization on metaphase preparations of human lymphocytes with ^{3}H-labeled PAI1 cDNA. As seen from Fig. 3 *Left*, chromosome 7 was the only chromosome that showed a grain number (36%) above background. The majority of the grains on chromosome 7 were associated with bands q21.3 and q22 of the long arm (29% of all grains observed, Fig. 3 *Right*), verifying and making more precise the localization obtained by the cell hybrid analysis.

RFLP. DNA from four unrelated individuals was digested with a variety of different restriction endonucleases (*Apa* I, *Bgl* II, *Msp* I, *Eco*RV, *Pvu* II, *Xmn* I, *Sac* I, *Bst*EII, *Bcl* I, *Taq* I, *Eco*RI, *Bam*HI, *Pst* I, *Hin*dIII, and *Nci* I) and analyzed by Southern blotting with the PAI1 cDNA probe. Digestion with *Hin*dIII revealed allelic fragments of 22 kbp (A1) and 18 kbp (A2). A representative autoradiograph of the segregation of the RFLP in a family is shown in Fig. 4. The segregation pattern obtained in all of the nuclear families, and both pedigrees, was consistent with Mendelian inheritance. The frequencies for A1 and A2 were 0.58 ± 0.05 and 0.42 ± 0.05, respectively, in a sample of DNA preparations accounting for 86 unrelated chromosomes representative of a general North American population. Because of the nearly optimal ratios of the major and minor allele, the polymorphism has a high polymorphism information content (0.369). An additional but much less frequent RFLP was found after *Xmn* I digestion. No polymorphisms were detected for the remaining restriction enzymes that were tested.

Linkage Analysis. The *Hin*dIII polymorphism detected by the PAI1 probe was used to test for linkage between *PAI1* and the *CF* locus and other markers previously mapped to this region of the long arm of chromosome 7. The segregation pattern of the *Hin*dIII RFLP was analyzed by Southern blot analysis using DNA prepared from each individual of the families described in *Materials and Methods*. Lod score

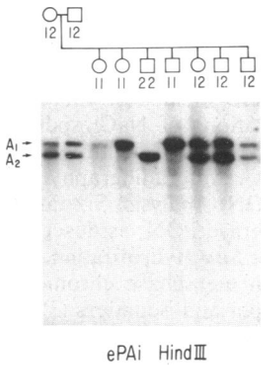

FIG. 4. Inheritance of *Hin*dIII RFLP. DNA samples were digested with *Hin*dIII and analyzed by Southern blotting with a 2.0-kbp fragment of the PAI1 cDNA probe. The family pedigree is shown above. The RFLP assignment of each individual is indicated below the pedigree. ePAi, pPAI1-A1 probe.

analysis of the cosegregation pattern of the CF disease phenotype and the RFLP, or the RFLP and additional markers assigned to chromosome 7q, was performed by using the LINKAGE program (see *Materials and Methods*).

The results of the two-point linkage analysis are shown in Table 1. Evidence was obtained for loose linkage between *PAI1* and the *CF* locus. The maximal likelihood estimate of the recombination distance (θ) between *PAI1* and *CF* was 0.20 [95% confidence interval, 0.10–0.36; with a maximal lod score (z) of 1.77 (odds ratio, 59:1)]. This suggested that the *PAI1* locus was located within the established CF linkage group on the long arm of chromosome 7, albeit somewhat distant from the *CF* locus. We therefore examined the linkage relationship between *PAI1* and other markers within this group. These included the loci for the c-*met* protooncogene (*MET*) (25), the erythropoietin gene (*EPO*) (28), and the gene for the serum protein paraoxonase (*PON*) (34). The results of these analyses (Table 1) demonstrated that *PAI1* is most

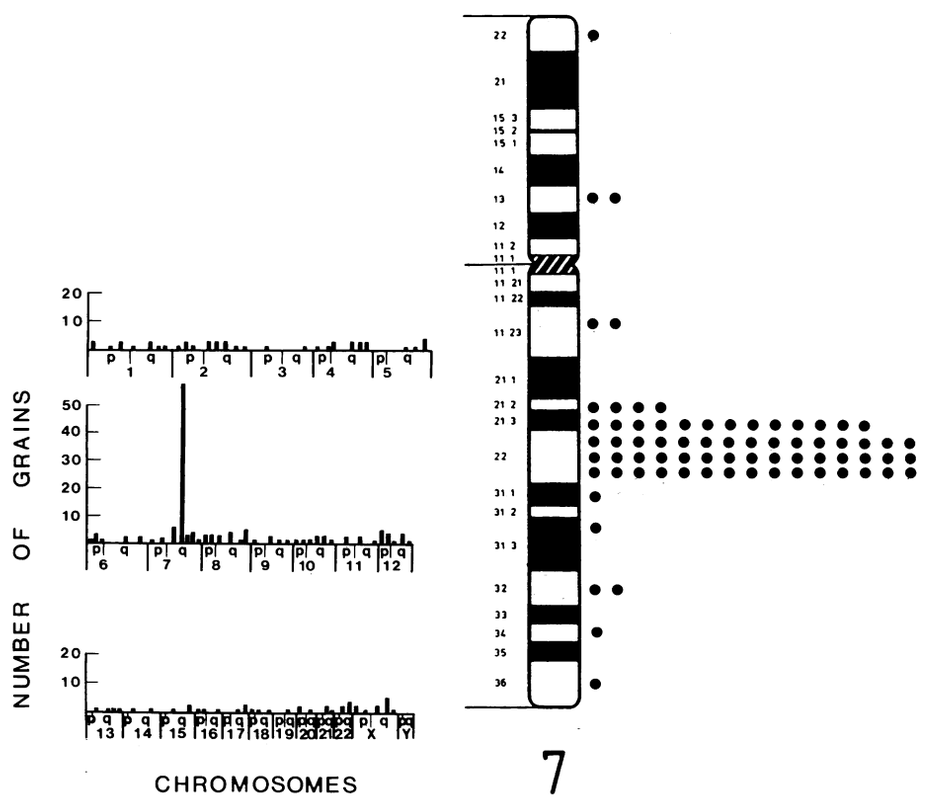

FIG. 3. Localization of human *PAI1* gene by *in situ* hybridization analysis. (*Left*) Histogram showing the grain distribution in 60 metaphases. Abscissa: chromosomes in their relative size proportions. Ordinate: number of silver grains. Overall, 203 grains were observed, of which 73 (36%) were on chromosome 7. Background grains were found distributed evenly on all chromosomes. (*Right*) Sublocalization of *PAI1* to 7q21.3-q22 (58 grains = 29% of all grains observed).

Table 1. Linkage analysis of gene loci for *PAI1* vs. *CF*, *EPO*, the c-*met* protooncogene (*MET*), and paraoxonase (*PON*)

Loci	Number of informative families	Lod (z) score at recombinant fraction (θ)							
		0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40
		$\hat{\theta}$ = 0.20; $\hat{z}$ = 1.77; 95% confidence interval = 0.10–0.36								
PAI1 vs. *CF*	13		−0.18	0.68	1.57	1.77	1.61	1.27	0.86	0.47
		$\hat{\theta}$ = 0.03; $\hat{z}$ = 10.08; 95% confidence interval = 0.001–0.11								
PAI1 vs. *EPO*	9		9.98	9.19	8.13	6.95	5.71	4.43	3.14	1.91
		$\hat{\theta}$ = 0.18; $\hat{z}$ = 2.23; 95% confidence interval = 0.10–0.33								
PAI1 vs. *MET*	18		−0.55	1.50	2.16	2.21	1.94	1.49	0.95	0.45
		$\hat{\theta}$ = 0.28; $\hat{z}$ = 0.80; 95% confidence interval = 0.10–0.45								
PAI1 vs. *PON*	4		−1.38	−0.18	0.38	0.66	0.78	0.79	0.71	0.54

closely linked to *EPO*, with a maximal likelihood estimate for θ of 0.03 and a maximal lod score of 10.08 (odds ratio, 1.2×10^{10}:1). For linkage of *PAI1* with *PON* and *MET*, the maximal likelihood estimates for θ and z were 0.28 and 0.80 vs. 0.18 and 2.23, respectively.

A three-locus test was performed to establish the position of *PAI1* relative to *EPO* and *PON*. Of the families that were informative for each marker tested for linkage to *PAI1* (Table 1), two families were jointly informative for *PAI1*, *PON*, and *EPO*. Results of the three-locus analysis using data obtained from these families are shown in Fig. 5. In this analysis the distance between the *EPO* and *PON* loci was held constant, and the relative probability of the *PAI1* locus was calculated at the indicated intervals. The data are consistent with the order *PON*, *PAI1*, *EPO* with odds of 40:8:1 over the alternative orders *PON*, *EPO*, *PAI1* or *PAI1*, *PON*, *EPO*, respectively.

DISCUSSION

We assigned the gene for *PAI1* to 7cen-7q32 based on the pattern obtained when a human PAI1 cDNA was hybridized to a panel of DNAs isolated from mouse–human somatic cell hybrids, sublocalized to bands q21.3-q22 by *in situ* hybridization.

Analysis of the segregation of the *Hin*dIII RFLP in families previously typed for the loci *CF*, *MET*, *PON*, *COL1A2*, and *EPO* showed that the *PAI1* gene is part of this linkage group on the long arm of chromosome 7. The *PAI1* gene is closely linked to the *EPO* locus (estimated genetic distance, 3 cM), which recently was found to be tightly linked with the collagen locus *COL1A2*, with no evidence of recombination ($\hat{\theta} = 0, \hat{z} = 4.81$) (P.W., N.S., P.S., and K.W.K., unpublished data). On this basis, the 20 (10–36‡‡)-cM recombination distance of *PAI1* from *CF* found in this study is in good agreement with those of 11 (3–27*) and 16 (10–26*) cM found for *EPO* and *COL1A2*, respectively (P.W., N.S., P.S., and K.W.K., unpublished data; ref. 28). Previous multipoint linkage analysis has established the order (*EPO*, *COL1A2*), *PON*, (*MET*, *CF*) (P.W., N.S., P.S., and K.W.K., unpublished data), with *EPO*, *COL*, and *PON* centromeric to *CF*. According to the results of the three-point analysis in the present study, the most probable location of *PAI1* is between *EPO* and *PON*—i.e., (*EPO*, *COL1A2*), *PAI1*, *PON*, (*MET*, *CF*), with *PAI1* centromeric to *CF* (see above) and telomeric to *EPO*.

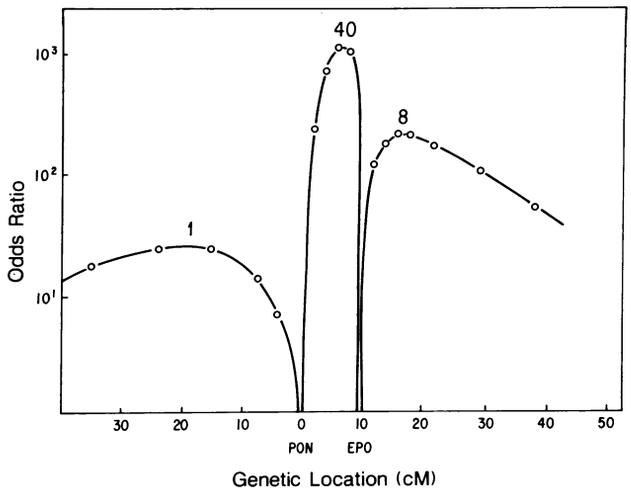

FIG. 5. Likelihood of the localization of the *PAI1* gene locus in relation to *PON* and *EPO*. Abscissa: genetic distance from PON. Ordinate: odds ratio for localization of *PAI1* at the distance indicated vs. localization of *PAI1* at infinite distance from *PON* (i.e., no linkage). The distance between *PON* and *EPO* is assumed to be 10 centimorgans (cM). The relative odds for the orders *PON*, *PAI1*, *EPO*: *PON*, *EPO*, *PAI1*: *PAI1*, *PON*, *EPO* are 40:8:1.

PAI1 may prove to be an important genetic marker. The polymorphism information content of 0.369 is high for a diallelic system; thus, the majority of families will be informative for gene mapping by linkage analysis.

The *PAI1* RLFP may be valuable in genetic analysis of plasminogen activation-related diseases, such as cancer and certain thromboembolic disorders. In preliminary studies, no rearrangements of the PAI1 sequences were found by Southern blotting either in 18 carcinoma samples or in 18 additional cell lines (R.W. and K.A., unpublished data). These included 12 colon carcinomas, 6 breast carcinomas, and tumor cell lines with previously demonstrated oncogene rearrangements and amplifications (42). However, these results must be interpreted with caution since small changes would have been missed due to the scale of the Southern blotting method and since we do not yet know the genomic organization of the PAI1 sequences. In relation to the role of plasminogen activation in the breakdown of matrix proteins in cancer, the close linkage between *PAI1* and the collagen locus *COL1A2* is particularly noteworthy because plasmin is an activator of latent collagenases (43, 44), and PAI1 may therefore serve to protect collagen.

Some thrombotic disorders, including myocardial infarction in young patients and recurrent deep vein thrombosis, are in some cases associated with increased plasma levels of PAI1 (45–47), and familial occurrence of venous thrombosis with high level of PAI1 has been reported (48, 49). The availability of the cloned gene sequence will allow molecular studies of the pathophysiology of these disorders, and the segregation of the RFLP in families with thrombosis will allow us to determine whether there is a genetic component involving the *PAI1* locus.

The excellent technical assistance of Helle Abildgaard, Kirsten Lund Jakobsen, Vivi Kielberg, Anna Margrethe Poulsen, Aita Haataja, and Irma Kurvinen is gratefully acknowledged. Determinations of PON serum activity were generously provided by Dr. B.

‡‡95% confidence interval.

LaDu (University of Michigan). We thank Drs. Robert Schwartz and Richard Doherty (Rochester, NY) for their role in identification and collection of members of the inbred pedigrees. This work was supported financially by the Danish Cancer Society, the Danish Medical Research Council, PF Ingegneria Genetica e Basi Molecolari delle Malattie Ereditarie and PF Oncologia, Consiglio Nazionale delle Ricerche, Italy, Finnish Cancer Foundation, and National Institutes of Health Grants HL31916 and AM36921.

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