KALLIKREIN PHENOTYPING BY ISOELECTRIC FOCUSING

H-J Leifheit[1] and H. Cleve[2]

1 Blood Transfusion Service, Bavarian Red Cross, Munich, FRG

2 Institute of Anthropology and Human Genetics, University of Munich, FRG

Introduction

The influence of kallikrein on the coagulation and the fibrinolytic system depends on the reciprocal activation with factor XII. The inactive prekallikrein exists as a single polypeptide chain of 619 amino acids with a molecular weight of 85.000. Activation by factor XIIa is achieved by the cleavage of an internal Arg-Ile bond. Plasma kallikrein is then composed of a heavy chain (371 amino acids) and light chain (248 amino acids) held together by disulfide bonds (1, 4, 5, 6). The aminoacid sequence of human plasma prekallikrein shows 58 % identity with factor XI (2, 3). The heavy chain is composed of repeated sequences homologous to factor XI. The light chain contains the catalytic part of the enzym and is homologous to the trypsin family of the serine proteases (1). In plasma prekallikrein circulates in a complex with high MW kininogen (5). The normal plasma concentration is 50µg/ml. Prekallikrein deficiency causes abnormalities in the coagulation, fibrinolytic, complement and kinin systems (7). Several different kallikrein genes are found. The nucleotid sequences show homologies (10). Several DNA polymorphisms in the kallikrein genes are observed (11). The human renal kallikrein and prostate specific antigen (PSA) genes were localized to chromosome 19q13 (8, 9).

Material and method

Neuraminidase treated and untreated serum and plasma samples from unrelated, healthy blood donors and from paternity cases were examined. For pI-determination marker proteins from 4.7 - 10.6 were used. Kallikrein antibodies were purchased from Behring (FRG) or donated by Dr. Müller-Esterl, University of Munich. Antibody specificities were tested with highly purified kallikrein and plasminogen isolated by Dr. Müller-Esterl (12).

Kallikrein was demonstrated by isoelectric focusing in 0.8 % agarose gels with the pH-range from 5 - 8. Immunolocical detection was done by immunofixation or immunoblotting.

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr
© Springer-Verlag Berlin Heidelberg 1990

Advances in Forensic Haemogenetics 3 (c) Springer-Verlag Berlin Heidelberg

Results

Fig 1 shows kallikrein phenotypes of untreated serum samples demonstrated with isoelectric focusing and immunoblotting. The band pattern is observed in the pH-range from 5.9 - 7.3. In the cathodal side of the band pattern the different phenotypes can be read off.

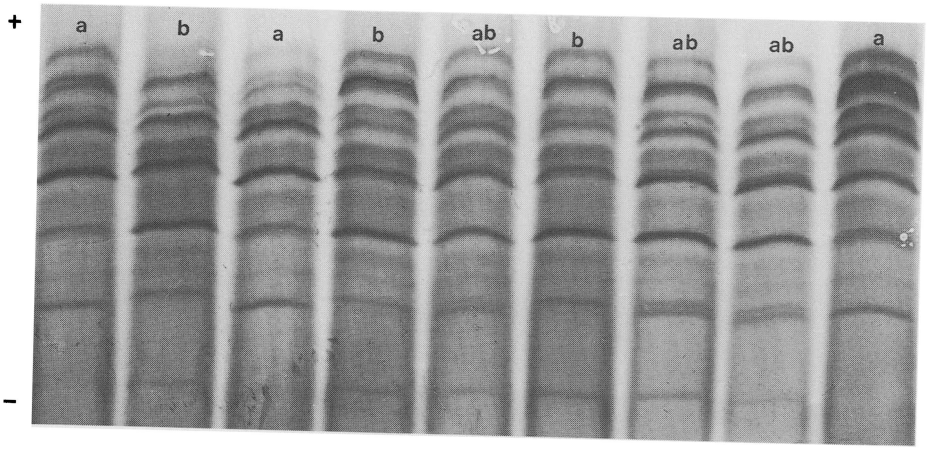

Fig. 1: Kallikrein phenotypes demonstrated by IEF and immunoblotting of neuraminidase untreated serum samples

Fig 2 demonstrates kallikrein after neuraminidase treatment. The band pattern is focused between the marker bands 6.4 - 8.3. Three common kallikrein phenotypes a, ab, b are observed. The neuraminidase treated as well as the untreated material shows a polymorphism correlated to plasminogen. The correlation does not depend as a cross reaction of the antibodies.

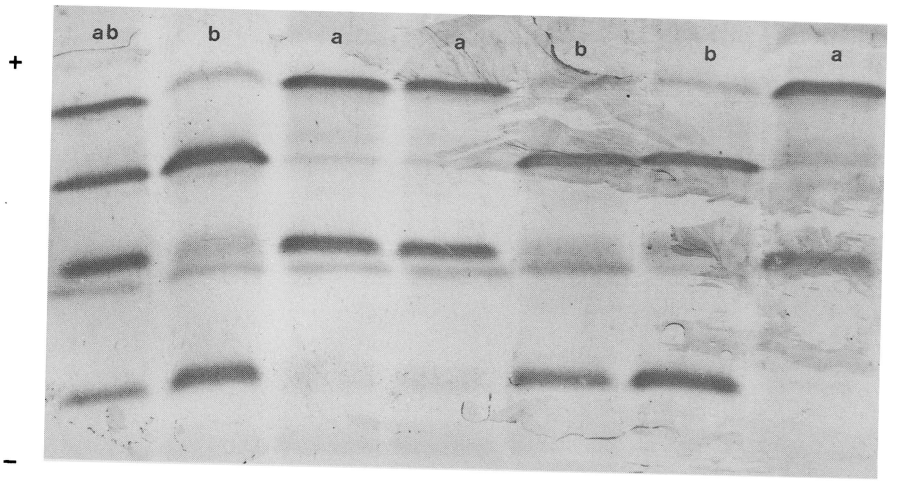

Fig. 2: Kallikrein phenotypes after neuraminidase treatment

Advances in Forensic Haemogenetics 3 (c) Springer-Verlag Berlin Heidelberg

Antibody specificity was tested with highly purified kallikrein and PLG. We did not observe immunological cross reactions (Fig 3).

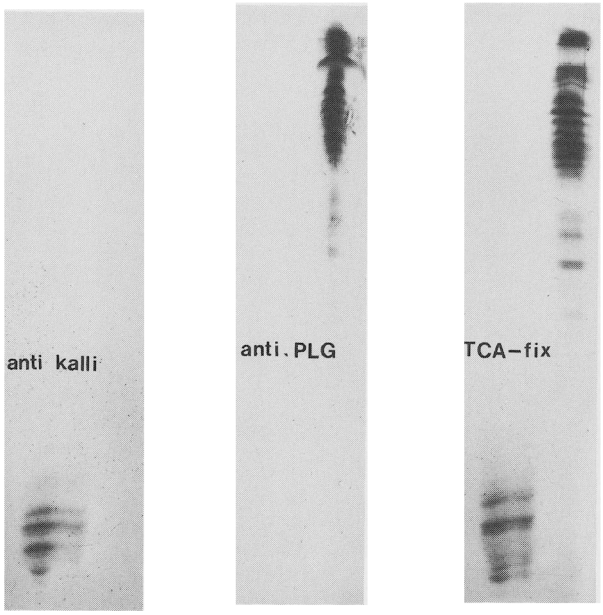

Fig. 3: Antibody specifity tested with purified PLG and kallikrein

The relationship between PLG- and kallikrein phenotypes as observed by IEF and immunological detection with blotting technique is shown in table 1. In some sera kallikrein band patterns are found which show additional bands not observed in PLG - a*, a*b (Fig.4).

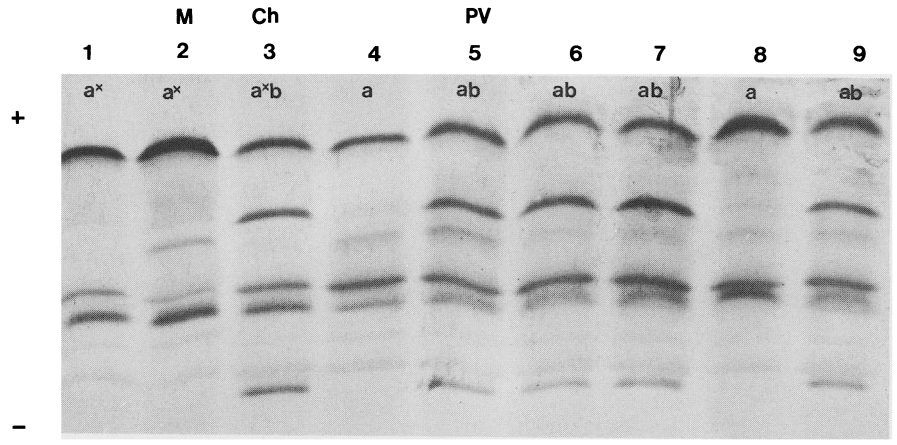

Fig. 4: Different kallikrein a phenotypes of neuraminidase treated sera. Paternity case 15 (Fig. 5) Lane 2, 3 and 5

Advances in Forensic Haemogenetics 3 (c) Springer-Verlag Berlin Heidelberg

Fig 5 shows the destribution of PLG and kallikrein phenotypes in 16 "families".

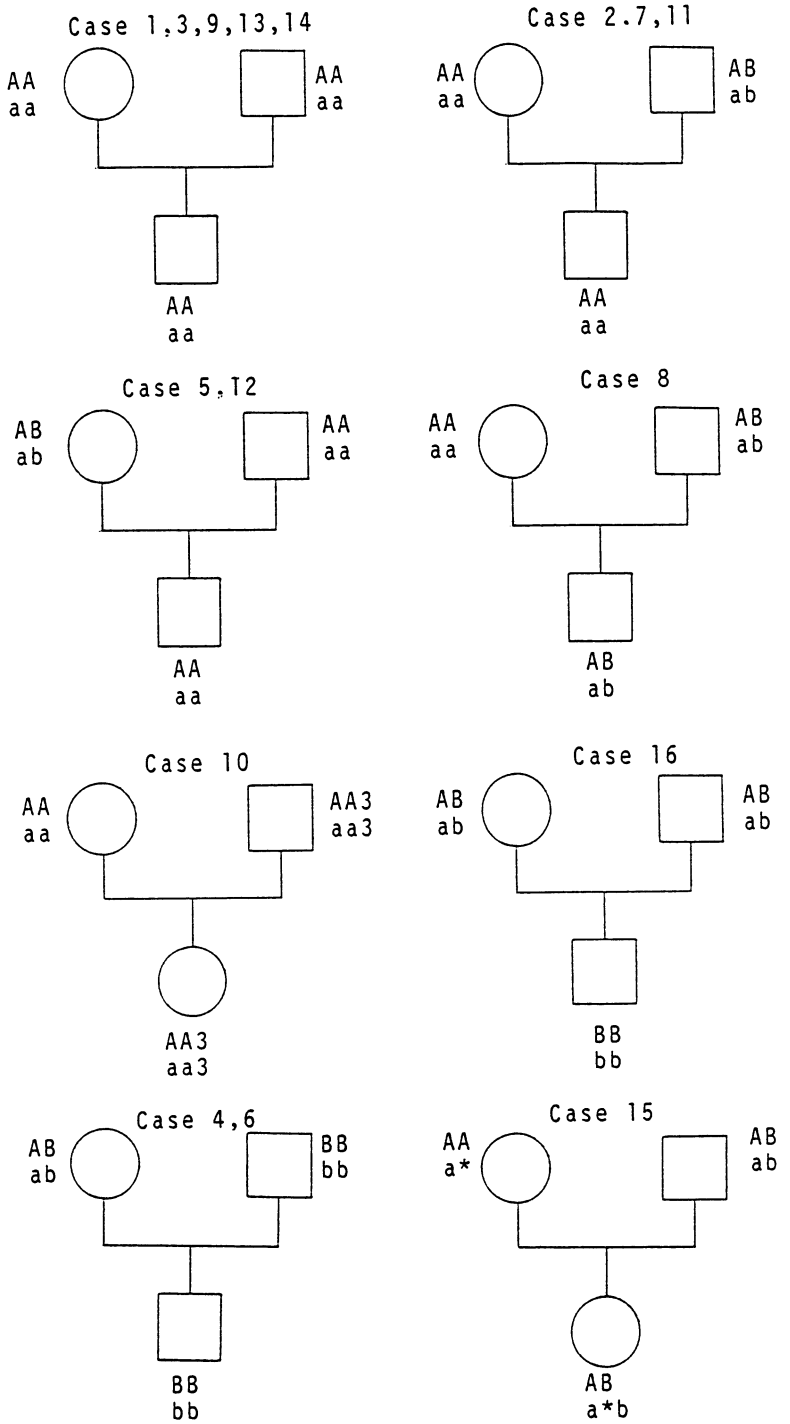

Fig. 5: Pedigrees of non excluded (> 99.9 %) paternity cases

Advances in Forensic Haemogenetics 3 (c) Springer-Verlag Berlin Heidelberg

Table 1

Relationship between PLG- and kallikrein phenotypes as examined by IEF

PLG		Kallikrein	
n	Phenotype	n	Phenotype
		111	a
119	A		
		8	a*
		86	ab
92	AB		
		6	a*b
18	B	18	b
3	AA3	3	aa3
1	BA3	1	ba3
1	BM2	1	bm2

Discussion

PLG and kallikrein are definitely not independent systems. But, on the other hand, there is not a complete correspondence of the band patterns of PLG and kallikrein. Additional bands in kallikrein were observed in particular with a.

The relationship between the two systems is unclear with the presently available results. A genetic interpretation of these results would suggest chromosomal linkage of the genes for PLG and kallikrein at the long arm of chromosom 6 with the PLG gene locus at 6q26-27 (13). Alternatively, a physiological interpretation could be entertained by the assumption of complex formation or other interactions.

Literature

1 Chung DW, Fujikawa K, McMullen BA, Davie EW 1986, Biochemistry 25, 2410
2 Heimark RL, Davie EW 1979, Biochemistry 18, 5743
3 Fujikawa K, Chung DW, Hendrickson LE, Davie EW 1986, Biochemistry 25, 2417
4 Wuepper KD, Cochrane CG 1972, J Exp Med 136, 1
5 Mandle RJ, Colman RW, Kaplan 1976, Proc Natl Acad Sci 11, 4179
6 Bouma BN, Miles LA, Beretta G, Griffin SH 1980, Biochemistry 19, 1151
7 Sollo DG, Salem A 1985, Am Clin Lab Sci 15, 279
8 Evans BA et al. 1988, Biochemistry 27, 3124
9 Sutherland GR et al. 1988, Cytogenet Cell Genet 48, 205
10 Morris BJ 1989, Clin Exp Pharmacol Physiol 16, 345
11 Baker AR, Shine J 1985, DNA 4, 445
12 Müller-Esterl W, Rauth G, Lottspeich F, Kellermann J, Henschen A 1985, Eur J Biochem 149, 15
13 Lindahl G, Gersdorf E, Menzel HJ, Duba C, Cleve H, Humphries S, Utermann G 1989, Hum Genet 81, 149

Advances in Forensic Haemogenetics 3 (c) Springer-Verlag Berlin Heidelberg

3 General Biostatistics

Advances in Forensic Haemogenetics 3 (c) Springer-Verlag Berlin Heidelberg

Advances in Forensic Haemogenetics 3 (c) Springer-Verlag Berlin Heidelberg