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Plasminogen (PLG): A Useful Genetic Marker for Paternity Examinations

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Summary. The genetically determined polymorphism of plasminogen (PLG) was analyzed by isoelectric focusing on polyacrylamide gels. For analysis neuraminidase-pretreated sera were used. PLG was developed functionally by activation with urokinase and subsequent lysis of casein in an agar overlay. In a random sample of 957 unrelated healthy individuals from Southern Germany, three common phenotypes, PLG1, 2–1, and 2, and five rare variants were found. The allele frequencies were: PLG*1 = 0.7174, PLG*2 = 0.2780, and PLG*Var = 0.0046. The theoretical exclusion rate in cases of disputed paternity is 16.5%.

Key words: Plasminogen (PLG), Genetic polymorphism – Paternity testing

Zusammenfassung. Der genetisch determinierte Polymorphismus des Plasminogens (PLG) wurde mit der isoelektrischen Fokussierung auf Polyacrylamidgelen untersucht. Für die Untersuchungen wurden Neuraminidase behandelte Serumproben verwendet. Der PLG-Nachweis erfolgte funktionell durch Urokinaseaktivierung und darauffolgender Lyse von Casein im Agar-Overlay. Bei einer Stichprobe von 957 nichtverwandten, gesunden Personen aus dem süddeutschen Raum wurden drei häufige Phänotypen PLG1, 2–1 und 2 sowie fünf seltene Varianten gefunden. Folgende Allelfrequenzen wurden ermittelt: PLG*1 = 0,7174, PLG*2 = 0,2780 und PLG*Var = 0,0046. Aufgrund unserer Untersuchungsergebnisse errechnet sich für das PLG-System eine theoretische Ausschlußchance von 16,5%.

Schlüsselwörter: Plasminogen (PLG), Genetischer Polymorphismus – Vaterschaftsuntersuchung

Plasminogen is a plasma protein which is activated to plasmin. An endogeneous, physiologically acting activator is produced by endothelial cells. Exogeneous

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activators are the enzyme urokinase, which is present in normal urine, and the streptococcal enzyme, streptokinase. Human plasminogen is a glycoprotein with a molecular weight of 91,000 daltons and is synthesized in the liver (Raum et al. 1980b). The plasma concentration in adults is on the average 12 mg/dl. In its native form, the protein consists of a single polypeptide chain, with glutamic acid as its aminoterminal amino acid (Collen and De Maeyer 1975; Heimburger 1980). By limited proteolysis it is converted to plasmin, which is an active serin protease capable of cleaving fibrin. Because of this fibrinolytic activity, plasmin is an important component of the coagulation system.

The genetic polymorphism of plasminogen (PLG) was first described by Hobart (1979) and by Raum et al. (1980a). Several populations have been examined since then, including Europeans, North Americans and Japanese (Mauff et al. 1981; Nishimukai et al. 1981; Ikemoto et al. 1982; Nakamura and Abe 1982; Spielmann and Kühnl 1982; Dykes et al. 1983).

In the present study, PLG polymorphism was investigated in a sample from Southern Germany. The distribution of phenotypes and alleles was determined, additional family data were obtained, and the usefullness of this marker system for paternity testing was confirmed.

Materials and Methods

Serum specimens were obtained from healthy blood donors and from persons involved in disputed paternity cases. The majority of individuals (more than 90%) were Germans, the remainder foreigners.

Sera were treated with neuraminidase from *Clostridium perfringens* by adding 10 µl of a 2 mg/ml enzyme solution to 100 µl serum and incubation at 37°C overnight.

Isoelectric focusing was carried out with flat bed gels of 250 × 115 × 0.5 mm. Gels were prepared with: 2.5 ml acrylamide solution, 28% (w/v); 2.5 ml bis-acrylamide solution, 2% (w/v); 0.8 ml ampholytes, pH 5–8; 0.1 ml ampholytes, pH 6–8 and 0.1 ml ampholytes, pH 3.5–9.5. After addition of 10 µl Temed and 600 µl ammonium persulfate solution, 1%, gels were polymerized for 30 min. Eight microliters of the sample was applied on filter paper pieces (Whatman No. 3) 3 cm from the anode. At the anode a mixture of 0.025 *M* aspartic acid and of 0.025 *M* glutamic acid was used; at the cathode a 0.5 *M* NaOH solution was employed. Isoelectric focusing was carried out in a Multiphor chamber (LKB 2117-301) at a cooling temperature of 8°C for 3 h with settings at 1600 V, 25 mA and 25 W.

PLG phenotypes were developed functional with an overlay method: 0.3 g casein and 0.12 g agar purum were dissolved in 20 ml Tris/HCl buffer at pH 8.3 and heated to 50°C; 1.5 mg urokinase (500 units) was added and the agar gel solution poured evenly over the polyacrylamide gel. After 3 h incubation at 37°C, PLG bands were made visible by fixation of the gel in 10% trichloroacetic acid for about 30 s.

In several gels plasminogen was identified immunologically by specific immunofixation. For the immunoprints monospecific antiserum against human PLG was used (Atlantic Antibodies purchased from Merz & Dade).

Results and Discussion

In Fig. 1 the banding patterns of nine different PLG phenotypes are presented, as obtained by isoelectric focusing on polyacrylamide gels. The patterns were developed after activation by urokinase and caseinolysis. The phenotypes of the

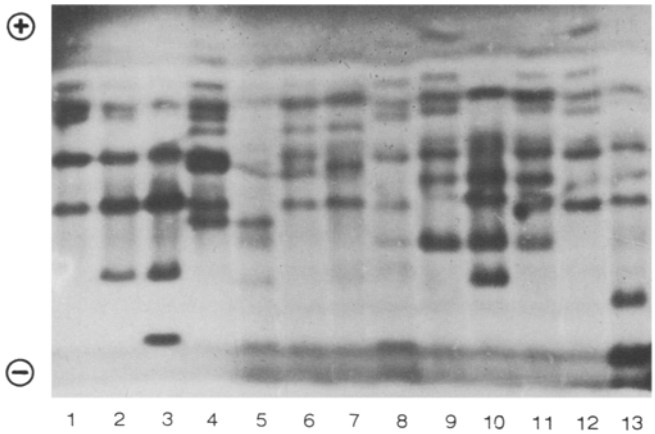

Fig. 1. PLG banding patterns after isoelectric focusing on polyacrylamide gels and urokinase activation in a caseinolytic overlay. Specimens have been treated with neuraminidase. From *left to right* the phenotypes are: (*1*) 1–1, (*2*) 2–1, (*3*) 2–2, (*4*) 1–M2, (*5*) 1–M2, (*6*) 1–A1, (*7*) 1–A2, (*8*) 1–M1, (*9*) 1–M1, (*10*) 2–M1, (*11*) *1–M1,* (*12*) 1–1, and (*13*) 1–B2. Samples 5 and 8 were kept in the refrigerator 4 weeks

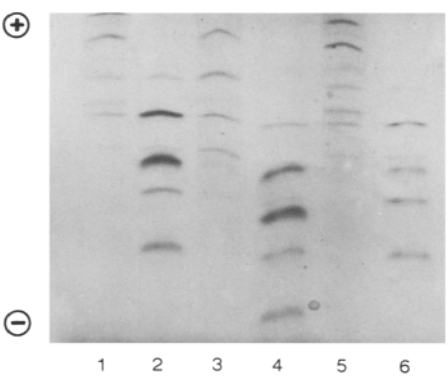

Fig. 2. Immunofixation pattern of plasminogen following isoelectric focusing of human serum samples. Specimens 2, 4 and 6 have been treated with neuraminidase. From left to right the phenotypes are: (*1*) 1–1, (*2*) 1–1, (*3*) 2–1, (*4*) 2–1, (*5*) 1–1, and (*6*) 1–1

homozygous PLG 1 and PLG 2 are characterized by two and three major bands, respectively, and additional anodally located minor bands. After neuraminidase pretreatment of sera, these phenotypes are clearly different. The heterozygous phenotype PLG 2–1 corresponds to a mixture of the two homozygous phenotypes. The nomenclature of the rare PLG variants is according to Mauff (1984). Figure 1 shows the PLG variants, A1, A2, M1, M2 and B2.

Apart from the dislocation of the PLG banding patterns to a more basic region, treatment with neuraminidase results in a finer resolution of the bands. In Fig. 2, the banding patterns of PLG 1 and PLG 2–1 are shown with and without neuraminidase treatment; the patterns were developed by specific immunofixation. The improved resolution after neuraminidase treatment is particularly useful for the differentiation of the PLG 2–1 and PLG 2 phenotypes.

In Table 1, the distribution of phenotypes and alleles is given in a random sample of 957 unrelated persons from Southern Germany. There is good agree-

Table 1. Distribution of PLG phenotypes and alleles in the Southern German population

		PLG phenotypes									
		1–1	2–1	2–2	1–M1	1–M2	2–M1	1–A1	1–A2	1–B	Total
Observed	(*n*)	487	391	70	3	2	1	1	1	1	957
	(%)	50.89	40.86	7.32	0.32	0.21	0.10	0.10	0.10	0.10	100.00
Expected	(*n*)	492.53	381.72	73.96			8.79	0.10	0.10	0.10	957.00
χ^2		0.062	0.226	0.212			0.005[a]				0.505

[a] Phenotypes with *n* (Exp) below 10 were combined for χ^2 calculation
Allele frequencies: PLG*1 = 0.7174, PLG*2 = 0.2780, and PLG*Var = 0.0046

Table 2. Comparison of PLG allele frequencies in several populations

Populations (authors)	*n*	Allele frequencies		
		PLG*1	PLG*2	PLG*V
English	327	0.710	0.290	0
Gambians	89	0.860	0.140	0
Hobart (1979)				
U.S. whites	102	0.686	0.299	0.015
U.S. blacks	127	0.795	0.193	0.012
U.S. orientals	69	0.964	0.029	0.007
Raum et al. (1980)				
Japanese				
Nishimukai et al. (1981)	258	0.958	0.020	0.022
Ikemoto et al. (1982)	400	0.969	0.031	0
Nakamura and Abe (1982)	750	0.956	0.011	0.033
Mexicans (lower social strata)	197	0.882	0.111	0.007
Mexicans (higher social strata)	123	0.858	0.110	0.032
Hummel et al. (1983)				
U.S. whites	1501	0.665	0.304	0.031
Dykes et al. (1983)				
Germans				
Mauff et al. (1981)	576	0.691	0.278	0.031
Spielmann and Kühnl (1982)	118	0.700	0.260	0.040
Jäger et al. (1983)	527	0.676	0.295	0.029
Osterhaus et al. (1984)	713	0.675	0.307	0.018
Weidinger et al. (1984)	658	0.724	0.272	0.004
This study	957	0.717	0.278	0.005

ment of the observed distribution with the distribution expected at population equilibrium. The frequencies for the alleles are: PLG*1 = 0.7174, PLG*2 = 0.2780, and PLG*Var = 0.0046.

In Table 2, the population data obtained by various authors are given. In the German, population allele frequencies for PLG*1 have been observed to vary between 0.675 and 0.724. These discrepancies may represent typing difficulties. The lower values may be caused by overestimation of the PLG 2 phenotype due to errors in the differentiation of PLG 2–1 and PLG 2. Differences in the distribution of PLG alleles in the various human populations are obvious: Blacks and Mexicans have higher frequencies for PLG*1 than Europeans; the highest frequency for PLG*1 is found in the Japanese with 0.969.

Table 3 presents family data from a total of 125 matings. There are no exceptions from the assumed autosomal codominant mode of inheritance.

In cases of disputed paternity, we have thus far observed 12 exclusions in the PLG system: three plaintiffs, four defendants, and five witnesses. These ex-

Table 3. Segregation of PLG types in 125 families with a total of 129 children

Matings	*n*	Children				
		1–1	2–1	2–2	1–A1	Total
1–1 × 1–1	31	31	—	—	—	31
1–1 × 2–1	49	21	28	—	—	49
1–1 × 2–2	8	—	10	—	—	10
2–1 × 2–1	24	7	14	5	—	26
2–1 × 2–2	9	—	6	3	—	9
2–2 × 2–2	1	—	—	1	—	1
1–1 × 1–A1	1	0	—	—	1	1
1–1 × 1–M2	1	1	—	—	—	1
2–1 × 1–M1	1	1	0	—	—	1
Total	125	61	58	9	1	129

clusions were confirmed in all cases by further exclusions in other blood groups, serum protein groups or enzyme systems. The theoretical exclusion rate with the PLG system alone was calculated to be 16.5%. The PLG system, therefore, appears to be a useful genetic marker, not only for population studies, but also for paternity testing.

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