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Polymorphism of Plasminogen in Tuscany (Italy)*

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Summary. The distribution of PLG phenotypes in the population of Tuscany (Central Italy) has been investigated by means of isoelectric focusing followed by immunofixation of desialyzed sera. In a random sample of 383 unrelated healthy blood donors registered at the Hospital of Pisa, three common phenotypes, PLG A, A–B, and B, and two rare variants were found. The allele frequencies calculated in our study were: PLG*A = 0.6749, PLG*B = 0.3225, and PLG*rare = 0.0026. The theoretical exclusion rate in cases of disputed paternity is 17.42%

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

Zusammenfassung. Die Verteilung der Plasminogen-Phänotypen in der Bevölkerung der Toskana wird untersucht. Die Bestimmung erfolgt an Neuraminidasebehandelten Seren nach isoelektrischer Fokussierung im Agarose-Gel und anschließender Immunofixation. In einer zufälligen Bevölkerungsstichprobe von 383 unverwandten gesunden Blutspendern des Krankenhauses Pisa wurden die drei neueren-Phänotypen PLG A, A–B und B gefunden sowie zwei seltene Varianten. Die berechneten Allelfrequenzen unserer Studie waren: PLG*A = 0,6749, PLG*B = 0,3225 und PLG* seltene Var. = 0,0026. Die theoretische Ausschließungschance im Vaterschaftsverfahren beträgt 17,42%.

Schlüsselwörter: Plasminogen (PLG), genetischer Polymorphismus – Vaterschaftsgutachten, Plasminogen

Introduction

Plasminogen is a component of the fibrinolytic system and is the precursor of the serine protease plasmin, which is formed by the action of specific activators.

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It is a plasmaprotein of the beta fraction, has a molecular weight of 91000 and a constant portion of 2%–3% carbohydrate, and is synthesized in the liver [1]. The genetic polymorphism has been demonstrated by different electrophoretic methods and by immunological or functional detection techniques. Hobart [2] and Raum et al. [3] were the first to describe two common codominant alleles (PLG*1, PLG*2). Since then, a large number of rare variants have been found in many population studies, making a uniform nomenclature indispensable for different designations of corresponding phenotypes [4]. It was suggested that the common alleles should be called PLG*A and PLG*B; the known variants with acid pI: PLG*A1 to *A3; intermediate variants: PLG*M1 to *M5; and basic variants: PLG*B1 to *B3.

The presence of a silent allele in the PLG system (PLG*Q0) has been demonstrated by family data and quantitative investigations [5–9].

Materials and methods

Serum samples were obtained from 383 unrelated blood donors registered at the Hospital of Pisa. Prior to use they were desialyzed by neuraminidase treatment (Boehringer) by overnight incubation at 37°C with 5 µl *Clostridium perfringens* neuroaminidase solution (1 mg/250 µl) in 50 µl serum.

The phenotypes were demonstrated using isoelectric focusing (IEF) in agarose gels (pH 3.5–9.5), followed by immunofixation according to the procedure suggested by Leifheit et al. [10] with minor modifications.

Gel casting was performed with flat bed gels (250 × 125 × 0.5 mm); 0.16 g agarose IEF, 2.0 g sorbit and 18.50 ml distilled water were dissolved in a flask and degassed. When the temperature had fallen to 75°C, 0.7 ml ampholine, pH 3.5–9.5 and 0.7 ml ampholine, pH 5.0–8.0 were added. Gels were polymerized for 30 min. After 1 h at room temperature gels were stored overnight at 4°C. Electrode solutions were 0.25 *M* acetic acid for the anode and 0.25 *M* NaOH for the cathode.

Prefocusing was carried out for 30 min in a Multiphor chamber (LKB 2117) at a cooling temperature of 8°C and 1200 V, 50 mA, 8 W.

Aliquots of 10 µl were then applied 1.5 cm from the anode, using Serva applicator strips. Electrophoretic conditions were 30 min salt run setting at 250 V and 90 min focusing setting at 1200 V.

After IEF, 1 ml anti human-plasminogen antiserum diluted 1/2 in saline (Atlantic Antibody) was spread over the gel surface and incubated for 90 min at 37°C. The gel was pressed for 20 min with a filter paper and washed in saline overnight.

After drying at 37°C, the gel was stained for 15 min with 0.2% Coomassie Brilliant Blue and destained in a solution of methanol 45% acetic acid 10%.

Results and discussion

Figure 1 shows the PLG phenotypes obtained by IEF on agarose gels with neuraminidase-treated serum samples followed by immunofixation with a monospecific PLG antiserum. The alleles PLG*A and PLG*B determine the three common phenotypes.

The distribution of phenotypes and the gene frequencies in our population sample for the PLG polymorphism are reported in Table 1. The distribution of observed and expected phenotype frequencies are in good agreement with the

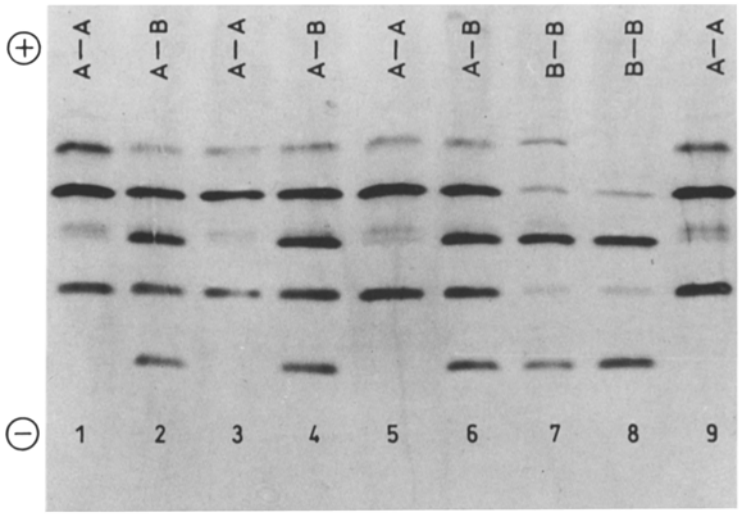

Fig. 1. PLG phenotypes observed by isoelectric focusing on agarose gel (pH range 3.5–9.5) and immunofixation. *Left* to *right*: *1*, A; *2*, AB; *3*, A; *4*, AB; *5*, A; *6*, AB; *7*, B; *8*, B; *9*, A

Table 1. Phenotype distribution and gene frequencies in a sample from Tuscany (Italy)

Phenotype	Observed		Expected		Gene frequencies
	n	%	*n*	%	
A	169	44.13	174.47	45.55	F*A = 0.6749
A–B	177	46.21	166.71	43.53	F*B = 0.3225
B	35	9.14	39.82	10.40	F*R = 0.0026
A–rare	2	0.52	1.35	0.35	
B–rare	0	0.00	0.64	0.17	
Rare	0	0.00	0.00	0.00	
Total	383	100.00	383.00	100.00	

Chi-square: 1.62 (B+rare), 1 *df*; $P > 0.10$

Hardy-Weinberg equilibrium. The estimated allele frequencies from our population sample are: PLG*A = 0.6749, PLG*B = 0.3225, PLG*Rare = 0.0026.

In Table 2 our allele frequencies are compared with those found in some other European population.

The frequency of PLG alleles in our study is similar to that found in North and Central Europe, but lower than that found in previous studies of other Italian populations.

PLG polymorphism is now used in several laboratories for parentage testing [11–14]. The theoretical exclusion rate in cases of disputed paternity was calculated at 17.42% (class I, 7.94%; class II, 9.48%). Apparent opposite homozygosity in family studies may be due to the occurrence of PLG*Q0 allele; its frequency has been variously estimated at 0.0013 [9] and 0.0035 [7].

Table 2. Geographic distribution of PLG gene frequencies in Europe

Population	*n*	PLG*A	PLG*B	PLG*rare	References
Denmark	1664	0.639	0.303	0.058	[15]
Germany					
North	604	0.713	0.270	0.018	[16]
South	380	0.682	0.297	0.021	[9]
West	1330	0.702	0.277	0.021	[14]
Switzerland	308	0.688	0.281	0.031	[17]
Italy					
Venetia	1325	0.840	0.158	0.001	[18]
Venetia Julia	716	0.858	0.140	0.001	[19]
Tuscany	[a]	0.83	0.16	0.01	[20]
Tuscany	383	0.675	0.322	0.003	This study
Latium	[a]	0.81	0.18	0.01	[20]
Campania	[a]	0.81	0.18	0.01	[20]
Lucania	[a]	0.78	0.21	0.01	[20]
South	287	0.766	0.214	0.021	[21]
Spain	703	0.800	0.200	0.000	[22]

[a] Tuscany + Latium + Lucania + Campania: $n = 2116$

The evidence for a null allele was provided by quantitative analysis: radial immunodiffusion [6–9] and electroimmunodiffusion [8]. Nevertheless, Scherz et al. [5] found it somewhat difficult to interpret some cases of opposite homozigosities by determination of the plasminogen concentration.

Inverse homozygosity between parents and their offspring may also be ascribed to the well-known technological difficulties in recognizing the anodal PLG variants [8].

References

1. Raum D, Marcus D, Alper CA, Levey R, Taylor PD, Starzl TE (1980) Synthesis of human plasminogen by the liver. Science 208:1036–1037
2. Hobart MJ (1979) Genetic polymorphism of human plasminogen. Ann Hum Genet 42: 419–423
3. Raum D, Marcus D, Alper CA (1980) Genetic polymorphism of human plasminogen. Am J Hum Genet 32:681–689
4. Skoda U, Bertrams J, Dykes D, Elberg H, Hobart M, Hummel K, Kuhnl P, Mauff G, Nakamura S, Nishimukai H, Raum D, Tokunaga K, Weidinger S (1986) Proposal for the nomenclature of human plasminogen (PLG) polymorphism. Vox Sang 51:244–248
5. Scherz R, Rohner R, Pflugshaupt R, Butler R (1986) Genetic polymorphism of plasminogen in the Swiss population. Adv Forensic Haematol 1:279–281
6. Brandt-Casadevall C, Dimo-Simonin N, Gujer HR (1987) A plasminogen silent allele detected in a Swiss family. Hum Hered 37:389–391
7. Dykes DD, Polesky HF (1988) Incidence of the PLG*Q0 allele in human populations. Adv Forensic Haematol 2:261–264

8. Skoda U, Goldmann SF, Handler SC, Hummel K, Lechler E, Lubcke I, Mauff G, Meyer-Bornecke D, Pesch S, Pulverer G (1988) Plasminogen hemizygosity. Vox Sang 54:210–214
9. Weidinger S, Patutschnick W, Schwarzfischer F (1988) Further evidence of a silent plasminogen (PLG) allele in two paternity cases. Z Rechtsmed 101:99–104
10. Leifheit HJ, Gathof AG, Cleve H (1987) Plasminogen (PLG)-Typisierung mittels isoelektrischer Fokussierung auf Agarose-Gelen und Immunfixation. Artzl Lab 33:10–12
11. Mauff G, Erfurdt U, Pulverer G (1981) The application of human plasminogen (PLG) polymorphism to paternity testing. (Abstract) Ninth International Congress of the Society for Forensic Haemogenetics, Berne, 29.9.1981–8.10.1981
12. Dykes DD, Mount M, Polesky HF (1984) Parentage testing using the serum protein plasminogen (PLG). Am J Clin Pathol 82:722–725
13. Weidinger S, Schwarzfischer F, Muller H, Cleve H (1985) Plasminogen (PLG): A useful genetic marker for paternity examinations. Z Rechtsmed 94:165–171
14. Skoda U, Klein A, Lubcke I, Mauff G, Pulverer G (1988) Application of plasminogen polymorphism to forensic hemogenetics. Electrophoresis 9:422–426
15. Eiberg H, Mohr J, Nielsen LS (1981) Genetics and linkage relations of plasminogen. Clin Genet 19:5
16. Simeoni E (1985) Zum Nachweis von Plasminogen (PLG), Phänotypenverteilung und Genfrequenzen in Schleswig-Holstein, Spurenuntersuchungen. Beitr Gerichtl Med 43: 249–254
17. Dimo-Simonin N, Brandt-Casadevall C, Gujer HR (1985) Gene frequencies of plasminogen in Switzerland. Hum Hered 35:343–345
18. Cortivo C, Caenazzo L, Crestani C, Scorretti C, Benciolini P, Pornaro E (1986) The polymorphism of plasminogen (PLG) by ultrathin-layer isoelectric focusing. Z Rechstmed 96:275–278
19. Foi A, Michelon C, Scalettaris U (1988) Determinazione delle frequenze geniche e dei fenotipi del plasminogeno (PLG) nella provincia di Udine mediante isoelettrofocallizzazione su gel di poliacrilamide. Riv It Med Leg 10:545–549
20. Pascali VL, Ranalletta D, Gentile V, Fiori A (1984) Plasminogen allotypes and their use in paternity investigation. J Forensic Sci Soc 24:437
21. Pascali VL, Lucchini R, Petrucci P, Auconi P (1984) Abstracts of the 18th Congress of the International Society of Blood Transfusion, Munich 1984. Karger, Basel, p 184 (cited in [14])
22. Carracedo A, Concheiro L, Rodriguez Calvo MS, Montiel MD (1987) Plasma protein and red cell enzyme groups in Galicia (North West Spain). Z Rechtsmed 98:133–140