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Further Evidence of a Silent Plasminogen (PLG) Allele in Two Paternity Cases*

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Summary. Routine paternity testing has yielded two different cases of an apparent inverse homozygosity in the plasminogen (PLG) system. In one case, the child presented the phenotype PLG A and his putative father the type PLG B. The alleged father could not be excluded from the paternity in 25 additional blood group marker systems (biostatistical probability of paternity $W > 99.75\%$). In the other case an incompatibility was found in a mother-child pair. Analysis of PLG was carried out by isoelectric focusing on neuraminidase-treated sera. In both cases the immunologic and functional detection showed weaker banding pattern of the affected PLG types. The assumption of a silent allele in the PLG system was confirmed by quantitative investigations. The allele frequency of PLG * Q0 in the South German population was estimated to be 0.0013. In the same sample the variant PLG A3 has been shown to be polymorphic.

Key words: Plasminogen (PLG), silent allele – Paternity testing, plasminogen

Zusammenfassung. Bei Routineuntersuchungen der Vaterschaftsbegutachtung wurde in zwei verschiedenen Fällen eine entgegengesetzte Reinerbigkeit im Plasminogensystem (PLG) gefunden. In einem Fall zeigte das Kind den Phänotyp PLG A und der Putativvater den Typ PLG B. Der angebliche Vater konnte in 25 weiteren blutgruppenserologischen Merkmalsystemen nicht von der Vaterschaft ausgeschlossen werden (biostatistische Wahrscheinlichkeit für die Vaterschaft $W > 99.75\%$). Im anderen Fall wurde eine

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Inkompatibilität zwischen Mutter und Kind festgestellt. Die PLG-Untersuchung erfolgte mit der isoelektrischen Fokussierung an Neuraminidase-behandelten Serumproben. Bei der immunologischen und funktionellen Detektion war in beiden Fällen das Bandenmuster der betroffenen PLG-Typen geringfügig abgeschwächt. Die Annahme eines stummen Allels im PLG-System wurde durch quantitative Untersuchungen bekräftigt. Für die süddeutsche Bevölkerung wurde eine Allelfrequenz PLG * Q0 = 0.0013 errechnet. In derselben Stichprobe erwies sich die PLG A3-Variante als polymorph.

Schlüsselwörter: Plasminogen (PLG), stummes Allel – Vaterschaftsbegutachtung, Plasminogen

Introduction

Using isoelectric focusing the genetic polymorphism of human plasminogen (PLG) has been demonstrated independently by Hobart (1979) and Raum et al. (1980) on plasma and serum samples. Besides two common alleles, PLG * A and PLG * B, a number of rare variants have been found in many population studies. Different designations of corresponding phenotypes in the PLG system have made a uniform nomenclature indispensable (Skoda et al. 1986). It is known that PLG is a useful genetic marker for paternity testing (Mauff et al. 1981; Weidinger et al. 1985). A silent PLG * Q0 allele has been observed so far only in a few cases (Scherz et al. 1986; Brandt-Casadevall et al. 1987; Dykes and Polesky 1988; Skoda et al. 1988). The gene locus has recently been mapped to the long arm of chromosome 6 (Murray et al. 1987).

This report gives the results of PLG phenotyping in a sample from southern Germany. Further evidence for a silent PLG allele will be described in two cases of disputed paternities.

Materials and Methods

Serum samples were obtained from individuals involved in cases of disputed paternities. Prior to use they were desialyzed with neuraminidase (Boehringer) by overnight incubation at 37°C of 10 µl Clostridium perfringens neuraminidase solution (1 mg/500 µl) in 50 µl serum.

Isoelectric focusing (IEF) was carried out on thin-layer (0.5 mm) polyacrylamide and agarose gels. Polyacrylamide gels (250 × 115 × 0.5 mm) consisted of 9 ml stock solution (9.7 g/dl acrylamide and 0.3 g/dl bis-acrylamide), 1.1 ml Pharmalyte pH 5–8, 0.2 ml Pharmalyte pH 6.5–9.0, 2.4 ml glycerol (87%), and 5.3 ml deionized water. After degasing 6 µl TEMED and 180 µl ammonium persulfate (36 mg/ml) were added for polymerization. For analysis serum samples were applied by saturating Whatman no. 3 filter paper pieces (5 × 10 mm) placed 3 cm from the anodal strip. Electrode solutions were 0.5 M NaOH for the cathode and a mixture of 0.025 M aspartic acid and 0.025 M glutamic acid for the anode. IEF was performed in a LKB Multiphor chamber for 4 h with maximum settings at 2000 V, 20 mA, and 20 W. The cooling temperature was 8°C.

IEF on agarose gel (pH range of 3.5–9.5) and immunologic detection was carried out according to the method of Leifheit et al. (1987). The functional detection was carried out on polyacrylamide gel with a caseinolytic overlay (Weidinger et al. 1985). For quantitative determination Partigen plates from Behringwerke AG were used.

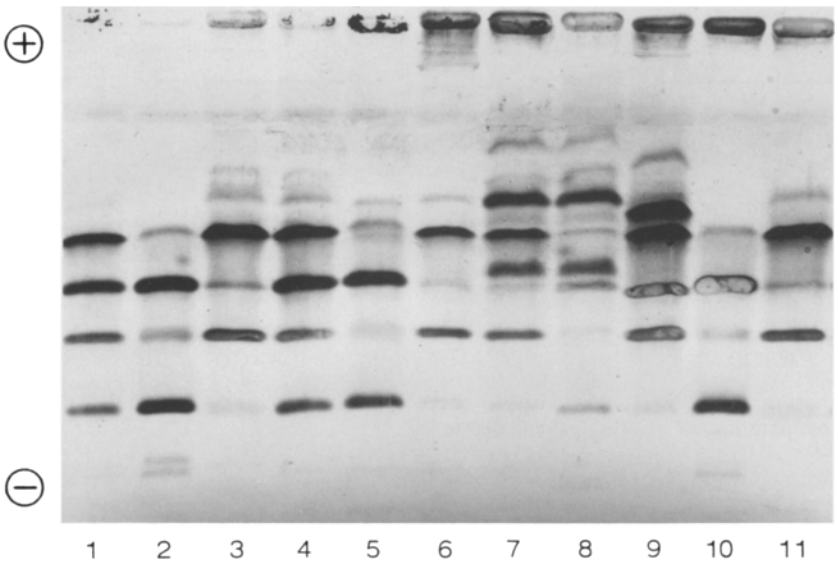

Fig. 1. PLG phenotypes observed by isoelectric focusing on agarose gel (pH range 3.5–9.5) and immunofixation. From *left* to *right*: (1) AB, (2) B, (3) A, (4) AB, (5) B-Q0, (6) A-Q0, (7) AA3, (8) BA3, (9) AA1, (10) B, and (11) A

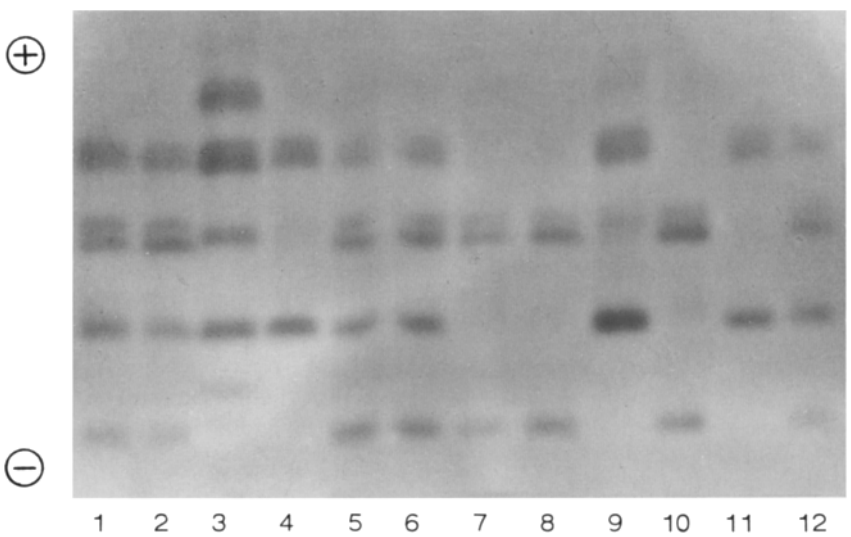

Fig. 2. PLG phenotypes observed by isoelectric focusing on polyacrylamide gel (pH range 5–9) and functional detection. From *left* to *right*: (1) AB, (2) AB, (3) AA3, (4) A, (5) AB, (6) AB, (7) B-Q0, (8) B, (9) A, (10) B, (11) A-Q0, and (12) AB

Results and Discussion

Figures 1 and 2 show patterns of several PLG phenotypes as obtained by IEF on agarose and polyacrylamide gels with neuraminidase-treated serum samples. For immunologic and functional detection a monospecific PLG antiserum and

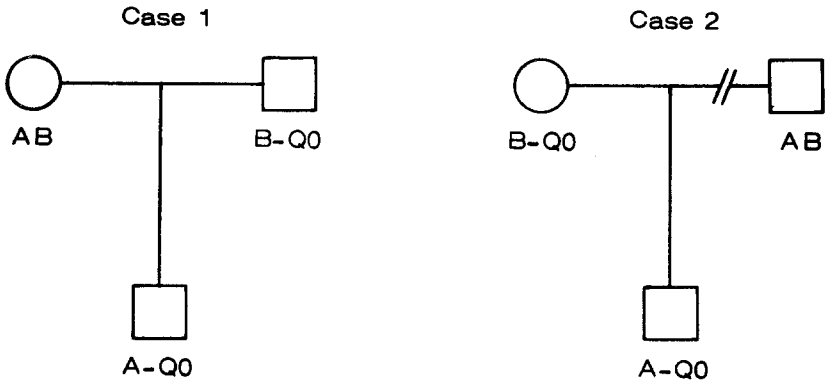

Fig. 3. Transmission of PLG*Q0 in two cases of paternity. In case 2 the alleged father was excluded from paternity in six other genetic markers

a caseinolytic overlay were applied. By using these methods an apparent incompatibility in the PLG system was found in two cases of disputed paternities (Fig. 3). Case 1 apparently shows inverse homozygosity between the child and the alleged father. The child originally had been classified as PLG A (Fig. 1, no. 6 and Fig. 2, no. 11), the father as PLG B (Fig. 1, no. 5 and Fig. 2, no. 7). The same apparent inverse phenotypic constellation has also been observed in a mother-child pair (see case 2). As shown in Figs. 1 and 2, the banding patterns of the affected individuals with PLG A and PLG B in case 1 were slightly weaker in comparison to normal phenotypes. In 25 additional blood group marker systems the alleged father could not be excluded as the father of this child (Table 1). The biostatistical probability for the paternity was $W > 99.75\%$ (Hummel 1986). Quantitative determinations of PLG by single radial immunodiffusion have shown approximately 50% of the normal concentration in the sera of the father-child pair. The father had 6.0 mg/dl and the child 5.6 mg/dl plasminogen, respectively. Values of normal phenotypes were between 10.0 and 15.5 mg/dl. These findings supported our earlier hypothesis of the existence of a silent allele in the PLG system (Weidinger and Schwarzfischer 1987). Similar results were found in case 2 for the mother and her child. In both cases the affected individuals were heterozygous for the so-called PLG * Q0 allele.

The distribution of PLG phenotypes and alleles in a sample of 380 unrelated individuals from southern Germany is given in Table 2. The observed phenotypes are in agreement with the expected number of phenotypes according to the Hardy-Weinberg equilibrium. There is also good general agreement with data observed in other European populations (Weidinger et al. 1985). In contrast to the former population study, we have found a relatively high frequency for PLG * A3 = 0.0146. This anodal variant seems to be polymorphic in the present study. We believe, therefore, that an improved resolution and a sensitive detection is necessary for the differentiation of PLG A3. The frequency of the silent allele PLG * Q0 was estimated to be 0.0013. In a large number of paternity cases the existence of PLG * Q0 (= 0.0035) has been sup-

Table 1. Phenotypes of 26 marker systems in a case of disputed paternity

	ABO	MNSs	Rh	K	Fy	Jk	Lu	Gm	Inv	HP	GC	TF	PI
Mother	A1	MNSs	ccdee	Kk	a+b+	a+b−	a−b+	1+2+10+	1−	2	1S	1	M1M3
Child	O	MNSS	CcDee	Kk	a−b+	a+b+	a−b+	1+2+10−	1−	2	2−1S	1	M3
Alleged father	O	MSs	CcDee	kk	a−b+	a+b+	a−b+	1+2+10+	1−	2	2	1	M1M3

	C3	BF	F13B	PLG	ACP1	PGM1	AK1	ADA	6PGD	GPT	ESD	GLO	GALT
Mother	S	F1S	1	AB	B	1A	1	1	A	1	1	2	N
Child	S	S	1	A–Q0	AB	1A	1	1	A	2–1	1	2	N
Alleged father	S	S	1	B–Q0	AB	1A	1	1	A	2–1	1	2	N

Table 2. Distribution of PLG phenotypes and PLG alleles in the population of southern Germany

		PLG phenotypes									
		A	AB	B	AA3	BA3	AA1	AM3	AM4	BQ0	Total
Observed	(*n*)	175	156	33	8	3	1	2	1	1	380
	(%)	46.05	41.05	8.68	2.12	0.80	0.26	0.52	0.26	0.26	100.00
Expected	(*n*)	176.54	154.01	33.59	7.56	3.30	0.67	1.35	0.67	0.67	378.36
	(%)	46.66	40.70	8.88	1.99	0.87	0.18	0.36	0.18	0.18	100.00
χ^2		0.013	0.026	0.010	0.228[a]						0.277

[a] Phenotypes with expected number below 10 were combined for χ^2 calculation

Allele frequencies: PLG*A = 0.6816, PLG*B = 0.2973, PLG*A3 = 0.0146, PLG*Var = 0.0052, and PLG*QO = 0.0013

ported by Dykes and Polesky (1988) The presence of a silent PLG allele should be considered in cases of disputed paternities if an exclusion constellation is noted only in this system.

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