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Plasminogen (PLG) polymorphism in northern Japanese: Confirmation of PLG*M6 allele

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Summary. Plasminogen (PLG) phenotyping has been performed on 450 unrelated individuals from northern Japan, using wide-scale ultrathin layer polyacrylamide gel isoelectric focusing combined with immunoblotting. One common phenotype and six rare ones were observed. The rare phenotypes included the recently detected allele PLG*M6 in a new combination with PLG*M5 allele. The estimated allele frequencies for PLG*A, PLG*A3, PLG*M2, PLG*M5, PLG*M6, PLG*B, and PLG*B2 were 0.961, 0.009, 0.001, 0.016, 0.001, 0.003, and 0.009, respectively.

Key words: Blood groups, plasminogen (PLG) polymorphism – Plasminogen polymorphism in northern Japanese

Zusammenfassung. Der genetische Polymorphismus des Plasminogen (PLG) wurde mit Hilfe der Isoelektrofokussierung und des Immunoblotting in einer Stichprobe von 450 nicht verwandten Personen aus der nördlichen Region Japans untersucht. Die folgende Allelfrequenzen wurden ermittelt: PLG*A = 0.961, PLG*A3 = 0.009, PLG*M2 = 0.001, PLG*M5 = 0.016, PLG*M6 = 0.001, PLG*B = 0.003 and PLG*B2 = 0.009.

Schlüsselwörter: Blutgruppen, Plasminogen (PLG) – Plasminogen-Polymorphismus, Nord-Japan

Introduction

The polymorphism of human plasminogen (PLG) locus is characterized by two common alleles and a number of rare variants. The common alleles are designated PLG*A and PLG*B, while the rare variants are differentiated into three groups according to their electrophoretic mobility (for details see [1, 2]).

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This study deals with plasminogen polymorphism in the Northern Japanese. The new PLG*M6 allele is ascertained.

Materials and methods

Plasma specimens were collected from 450 unrelated Japanese from the Miyagi prefecture in Northern Japan. Fresh samples were treated with neuraminidase by adding 5 µl of 1 U/ml enzyme to 20 µl plasma, and the mixture was incubated at 37°C overnight.

Isoelectric focusing in polyacrylamide gels of 0.2 mm thickness and 150 mm distance between the electrodes was employed. The gels contained 2.5% ampholines of pH ranges 3.5–10, 5–8, and 6–8 in 1:3:1 ratio and 12.5% sucrose, with gel concentration (T) = 5% and degree of cross linkage (C) = 3%. Riboflavin and UV light were used for the polymerization. The electrode solutions were 1 *M* phosphoric acid for the anode and 1 *M* ethanolamine for the cathode. The power unit was adjusted to provide an initial voltage of 300 V and maximum of 1400 V. After 40 min prefocusing, 5 µl neuraminidase treated plasma was applied 4 cm from the anode using 5 × 7 mm paper strips (Toyo no. 2). The paper strips were removed after 30 min and the total running time was 4 h at 2°C.

Immunoblotting was performed using a nitrocellulose filter (Bio-Rad, 0.45 µm) for passive transfer of proteins, 1:500 dilution in Tris-buffered saline (TBS) for anti-human plasminogen antibody (DAKO), and 1:800 dilution in TBS for peroxidase conjugated goat anti-rabbit IgG antibody (CAPPEL). The PLG pattern was visualized using 15 mg 4-chloro-1-naphthol dissolved in 1 ml acetone, then mixed with 40 ml TBS and 30 µl 20% hydrogen peroxide.

Results and discussion

Figure 1 shows the band pattern of the common and rare PLG phenotypes as obtained by electrofocusing in wide-scale ultrathin layer polyacrylamide gels and immunoblotting. The encountered PLG M6M5 phenotype confirms the PLG*M6 allele, which has recently been described but was unconfirmed [2]. This allele has previously been reported in a Japanese subject in heterozygote combination with PLG*A allele [3].

Distribution of PLG phenotypes and gene frequencies are presented in Table 1. The observed and expected phenotypes provide a good fit to the Hardy-Weinberg equilibrium. Our data are in accordance with those reported from

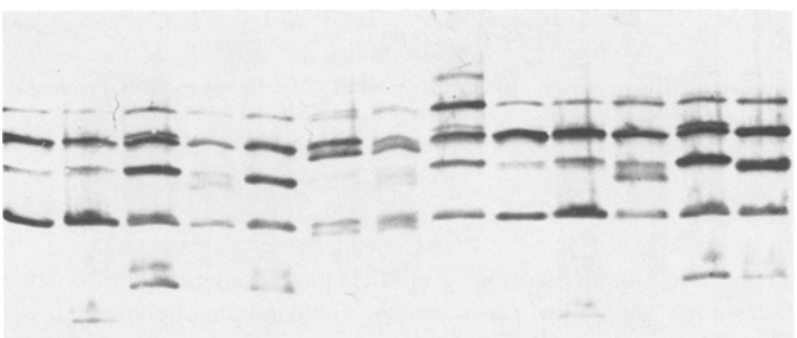

Fig. 1. PLG phenotypes after electrofocusing and immunoblotting of neuraminidase treated plasma. Anode at the *top*. From *left* to *right:* A, AB2, AM2, AB1(Ref.), AB, AM5, M6M5, A3A, A, AB2, AB1(Ref.), AM2 and AB

Table 1. PLG phenotypes and allele frequencies in Northern Japanese

Phenotypes	Number observed	%	Number expected	Allele frequencies
A	416	92.44	415.58	PLG*A = 0.961
A3A	8	1.78	7.78	PLG*A3 = 0.009
AM2	1	0.22	0.86	PLG*M2 = 0.001
AM5	13	2.89	13.84	PLG*M5 = 0.016
M6M5	1	0.22	0.01	PLG*M6 = 0.001
AB	3	0.67	2.59	PLG*B = 0.003
AB2	8	1.78	7.78	PLG*B2 = 0.009
Others	0	0.00	1.56	
Total	450	100.00	450.00	

$\sum\chi^2 = 0.059$, $df = 1$, $0.80 < P < 0.90$
Phenotypes with expected number below 10 were combined for χ^2 calculation

other areas of Japan [3–9] and confirm the existence of the new intermediate allele PLG*M6.

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