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Polymorphism of uridine monophosphate kinase: population study in Japanese and phenotyping in bloodstains

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Summary. The polymorphism of UMPK was investigated in 677 unrelated Japanese individuals. The allele frequencies were estimated to be UMPK*1 = 0.9594 and UMPK*2 = 0.0406. Our sample was devoid of the UMPK*3 allele. Phenotyping was also possible from bloodstains stored at 37°C for up to 1 week, at room temperature for up to 4 weeks and at 4°C over 10 weeks. The UMPK system can be a useful supplement for the mediocolegal grouping of bloodstains.

Key words: Polymorphism – Red cell enzyme – UMPK – Population study – Bloodstains

Zusammenfassung. Der Polymorphismus der Uridin Monophosphat Kinase (UMPK) wurde in einer Stichprobe von 677 nicht-verwandten Personen aus Japan untersucht. Folgende Allel-Frequenzen wurden ermittelt: UMPK*1 = 0,9594, UMPK*2 = 0,0406. Phänotypisierung war auch an Blutspuren möglich, welche eine Woche bei 37°C, 4 Wochen bei Zimmertemperatur oder mehr als 10 Wochen bei 4°C gelagert worden sind. Das UMPK System kann eine nützliche Ergänzung in der forensischen Blutspurenkunde sein.

Schlüsselwörter: Polymorphismus – Erythrozyten-Enzym – UMPK – Bevölkerungsstudie – Blutspuren

Introduction

Human red cell uridine monophosphate kinase (UMPK) exhibits genetic polymorphism with three codominant autosomal alleles UMPK*1, UMPK*2 and UMPK*3 (Giblett et al. 1974). Up to the present time, a number of population studies have been performed in various racial groups (see Table 2) and a rare variant allele UMPK*4 has been described in a Japanese population (Toyomasu et al. 1977). However, there has been no report on the application of this enzyme system to forensic bloodstain analysis. In the present study the distribution of UMPK types in a Japanese population was examined and the phenotyping in bloodstains was investigated for medicolegal use.

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Materials and methods

Blood samples were collected from 677 unrelated Japanese individuals living in Yamanashi Prefecture, a central part of Japan. Fresh hemolysates were prepared from red cells by freezing and thawing.

Blood samples from 20 individuals with known phenotypes were dropped onto filter paper (Toyoroshi No. 2, Tokyo, Japan) and allowed to dry for a few hours at room temperature. The bloodstains thus made were stored at 37°C in a thermostatic chamber, at room temperature and at 4°C and examined at 1-week intervals. The stains were cut in 3 × 8 mm pieces, treated with 10 µl of 0.05 *M* dithiothreitol (DTT, Sigma Chemical Co., USA) for 30 min at room temperature just before analysis.

UMPK types were determined by the method of Halasa et al. (1985) with slight modifications. Horizontal starch gel electrophoresis was performed using 10% Biotestgel (Biotest Serum Institut, Frankfurt, FRG). The gel buffer used was 0.005 *M* DL-histidine monohydrochloride/sodium hydroxide (pH 6.5); the bridge buffer was 0.136 *M* citric acid/sodium hydroxide (pH 6.5). Electrophoresis was carried out at a constant voltage of 6.5 V/cm for 5 h at 4°C.

After electrophoresis, the gel was horizontally sliced and stained for UMPK by the method of Giblett et al. (1974) using the

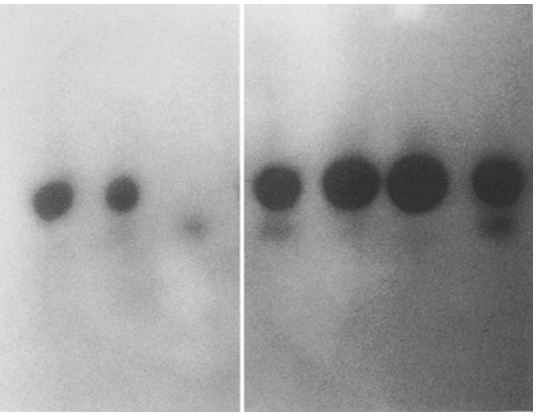

Fig. 1. Electrophoretic pattern of red cell UMPK types. The anode is at the top

Table 1. Distribution of UMPK types in Yamanashi Prefecture

Phenotype	No. observed (%)	No. expected
1	623 (92.02)	623.14
2–1	53 (7.83)	52.74
2	1 (0.15)	1.12
Total	677 (100.00)	677.00

Allele frequencies: UMPK*1 = 0.9594, UMPK*2 = 0.0406; χ^2 = 0.01; d.f. = 1; $0.90 < P < 0.95$

filter paper overlay technique. The gel was covered with a sheet of filter paper (Toyoroshi No. 2) soaked with 15 ml 0.1 *M* tris/hydrochloride acid buffer (pH 7.8) containing 26 mg uridine-5′-monophosphate (Sigma), 33 mg adenosine-5′-triphosphate (Boehringer Mannheim, FRG), 6.5 mg phosphoenolpyruvate (Boehringer), 9.5 mg NADH (Oriental Yeast Co., Tokyo, Japan), 75 mg magnesium chloride (Wako Pure Chemical Industries, Osaka, Japan), 260 mg potassium sulfate (Wako), 6 µl pyruvate kinase (Boehringer) and 35 µl LDH (Oriental). The gel was wrapped in a sheet of thin plastic film and incubated at 37°C for 60 min. The patterns were visualized and read under long-wave ultraviolet light.

Table 2. UMPK allele frequencies in various racial groups

Region	Population	No. tested	Allele frequency			Reference
			UMPK*1	UMPK*2	UMPK*3	
Asia	Japanese					
	Tokyo	635	0.9472	0.0528		Harada et al. 1975
	Yamanashi	677	0.9594	0.0406		Present study
	Mie	346	0.954	0.045	0.001	Kuwata and Ishimoto 1976
	Osaka	770	0.9694	0.0306		Toyomasu et al. 1977
	Negritos					
	Luzon	129	0.6550	0.3450		Omoto et al. 1978
	Malays					
	Kuala Lumpur	168	0.851	0.149		Zarinah et al. 1984
	Chinese					
	Kuala Lumpur	125	0.880	0.120		Zarinah et al. 1984
	Indians					
	Kuala Lumpur	121	0.942	0.058		Zarinah et al. 1984
	Kadazans					
	Sabah	256	0.959	0.041		Tan et al. 1979
Europe	Pole	462	0.9762	0.0238		Halasa et al. 1985
	German	351	0.949	0.051		Kuhn et al. 1975
	German					
	Dusseldorf	711	0.955	0.045		Driesel et al. 1982
	Schleswig-Holstein	1003	0.9505	0.0495		Sachs et al. 1988
	Swiss	220	0.9568	0.0432		Scheil and Scheffrahn 1987
	Italian					
	Rome	486	0.9722	0.0278		Ranzani et al. 1977
	Milan	429	0.9709	0.0291		Ranzani et al. 1977
	Spanish					
	Galicia	500	0.9550	0.0450		Caeiro et al. 1989
North America	Eskimos					
	Inupiat	267	0.788	0.019	0.193	Scott and Wright 1983
	Siberian Yupik	145	0.824	0.055	0.121	Scott and Wright 1983
	Central Yupik	1460	0.846	0.063	0.091	Scott and Wright 1983
	Sugpiaq	148	0.709	0.051	0.240	Scott and Wright 1983
	Aleut	162	0.697	0.065	0.238	Scott and Wright 1983
	Caucasian					
	Seattle	386	0.953	0.045	0.001	Giblett et al. 1974
	Afro-American					
	Seattle	92	0.989	0.011		Giblett et al. 1974
	Native African					
	Seattle	122	1.000			Giblett et al. 1974
	Cree Indians					
	Seattle	91	0.868	0.028	0.104	Giblett et al. 1974
	Oriental					
	Seattle	112	0.929	0.071		Giblett et al. 1974
South America	Venezuelan					
	Mestizo	442	0.979	0.020	0.001	Gallango and Suinaga 1978
	Warao Indians	64	0.914		0.086	Gallango and Suinaga 1978

Table 3. Positive results for the determination limits of UMPK types in 20 bloodstains stored at 37°C, room temperature and 4°C

Phenotype	No. tested	Temperature	Age of bloodstains (weeks)									
			1	2	3	4	5	6	7	8	9	10
1	14	37°C	14	14	14	0						
2–1	6		6	2	1	0						
1	14	Room temperature	14	14	14	14	14	0				
2–1	6		6	6	6	6	4	0				
1	14	4°C	14	14	14	14	14	14	14	14	14	14
2–1	6		6	6	6	6	6	6	6	6	6	6

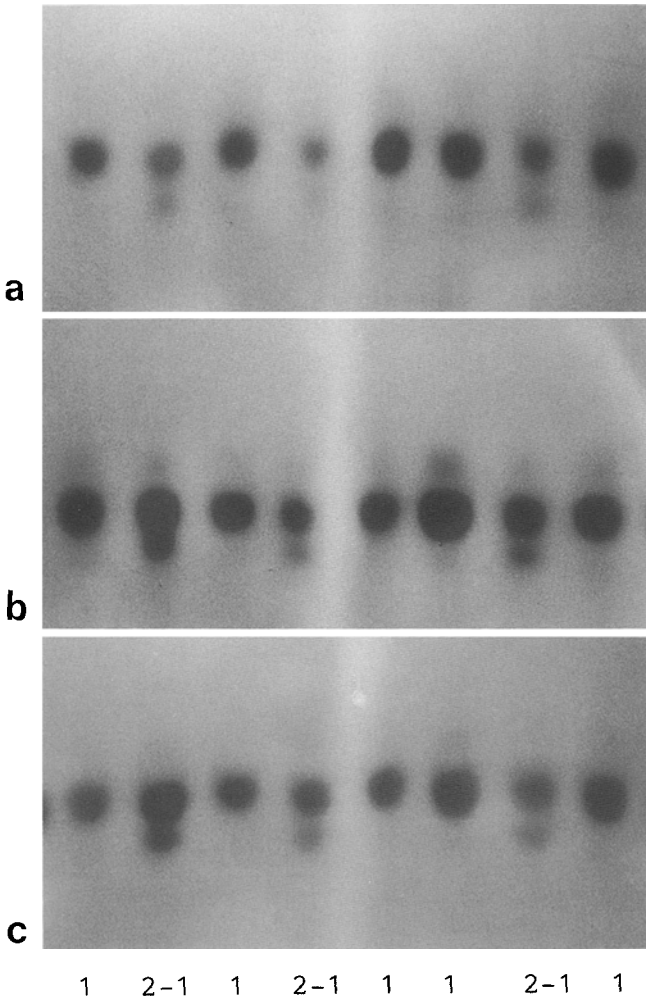

Fig. 2a–c. Electrophoretic patterns of UMPK types in bloodstains stored at 37°C for 1 week **a**, at room temperature for 2 weeks **b** and at 4°C for 4 weeks **c**. The anode is at the top

Results and discussion

Population study

In our sample three common phenotypes UMPK 1, 2–1 and 2 were demonstrated (Fig. 1), but neither the types associated with the UMPK*3 allele nor a rare variant phenotype was observed. The results for the distribution obtained from 677 individuals of Yamanashi Prefecture are given in Table 1. The allele frequencies were calculated to be UMPK*1 = 0.9594 and UMPK*2 = 0.0406. The observed numbers are in good agreement with the expectation according to the Hardy-Weinberg law.

Table 2 summarizes the UMPK allele frequencies reported for various racial groups. It is anthropologically interesting that the UMPK*3 allele is practically absent in Asian and European populations whereas it occurs with polymorphic frequency in Eskimos and American Indians. The lack of the UMPK*3 allele in the Japanese population disagrees with the generally accepted view that a long time ago the Mongoloids migrated via Siberia and Alaska to North and South America.

In conclusion, owing to the unbalanced allele proportion, UMPK may be of little utility value for paternity testing, but can be a useful genetic marker for human genetics and anthropological studies.

Phenotyping in bloodstains

The phenotypes UMPK 1 and 2–1 were well demonstrated also from dried and stored bloodstains. Table 3 presents the results for the determination limits of UMPK types in bloodstains stored at 37°C, room temperature and 4°C. All the bloodstains stored at 37°C were successfully typed for UMPK for periods of up to 1 week, at room temperature for periods of up to 4 weeks and at 4°C even for periods in excess of 10 weeks. With increasing time of storage, the bands of UMPK 2-1 became fainter and more indistinct than those of UMPK 1. In the present investigation the stability of the type 2 was not examined. Figure 2 shows the electrophoretic patterns of UMPK types in bloodstains under different storage conditions.

The above results show that this enzyme is stable enough to be used in medicolegal practice. The UMPK system would therefore provide a supplemental genetic marker for the grouping of bloodstains.

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