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PGM_1 Subtypes Determined by Agarose Gel Isoelectrofocusing

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Summary. PGM_1 subtypes were determined in red cell hemolysates by isoelectric focusing on agarose gel plates. By this modified procedure PGM_1 subtypes may be readily classified. Nine of the 10 expected phenotypes were found in a sample of 470 unrelated individuals from Southern Germany. The frequencies for the four alleles were found to be: $PGM_1^{1+} = 0.6212$, $PGM_1^{1-} = 0.1224$, $PGM_1^{2+} = 0.2043$, $PGM_1^{2-} = 0.0521$.

Key words: Blood groups, PGM_1-polymorphism – Phosphoglucomutase I, subtypes

Zusammenfassung. Der PGM_1-Polymorphismus wurde mit Hilfe der Isoelektrofokussierung auf Agarose-Gel-Platten untersucht. Mit dieser modifizierten Methode lassen sich die PGM_1-Untergruppen an Hämolysaten ohne Schwierigkeiten klassifizieren. Neun der erwarteten zehn Phänotypen wurden in einer Stichprobe von 470 nichtverwandten Personen aus Süddeutschland beobachtet. Folgende Allelhäufigkeiten wurden ermittelt: $PGM_1^{1+} = 0{,}6212$, $PGM_1^{1-} = 0{,}1224$, $PGM_1^{2+} = 0{,}2043$, $PGM_1^{2-} = 0{,}0521$.

Schlüsselwörter: Blutgruppen, PGM_1-Polymorphismus – Phosphoglucomutase I, Untergruppen

In 1964, Spencer et al. described first genetic variation of Phosphoglucomutase I (PGM_1). The three phenotypes PGM_1 1, PGM_1 2-1, and PGM_1 2 are determined by two autosomal alleles, PGM_1^1 and PGM_1^2 (Spencer et al. 1964). The PGM_1 locus is located on the short arm of chromosome 1 (Billardon et al. 1973; Douglas et al. 1973). With the use of polyacrylamide gel isoelectrofocusing (PAGIF) Bark et al. (1976), Sutton and Burgess (1978), Kühnl et al. (1977, 1978), and Welch et al. (1978) were able to distinguish 10 different PGM_1-subtypes. Acid starch gel electrophoresis led Bissbort et al. (1978) to a similar result. Genetically, these 10 phenotypes are apparently determined by four alleles at the PGM_1 locus (Kühnl et al. 1977).

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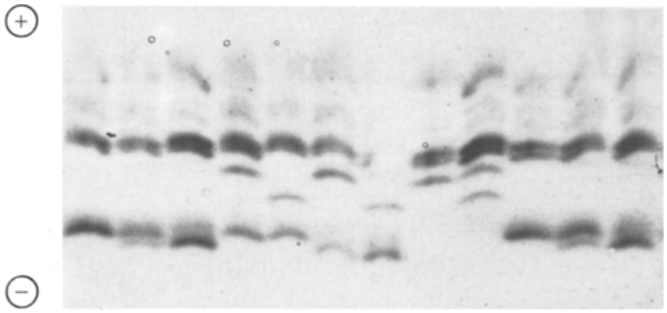

Fig. 1. Separation of PGM_1 isozymes after 175 min PAGIF (pH 4—6.5). From left to right are the observed common PGM_1 subtypes: 1+1+, 1+1−, 1−1−, 1+2+, 1+2−, 1−2+, 1−2−, 2+2+, 2+2−, 1+1+, 1+1−, and 1−1−

In this study, the PGM_1 polymorphism was analyzed in red cell hemolysates by a modified agarose gel isoelectrofocusing (AGIF) procedure.

Materials and Methods

Hemolysates of 470 unrelated healthy individuals from Southern Germany were examined. Erythrocytes were washed twice in physiological saline solution and subsequently hemolyzed by ultra-sonification (Sonifier). Agarose gel plates were prepared in the size $125 \times 260 \times 1$ mm. Agarose concentration was 0.8%, D-Sorbit was added to a final concentration of 10% (w/v). For the pH gradient, a mixture of Agarose-Ampholine pH 3.5—9.5 and Ampholine pH 5—7 was prepared; the final concentrations were 1.8% and 2.2%, respectively. Samples were applied with pieces of filter paper (5×7 mm) which were placed on the gel surface near the anode. A Multiphor chamber (LKB) was used. Electrofocusing was carried out with maximally 1100 V and 40 mA, stabilized at 16 W, for 90 min. The temperature was kept at +6° C by a circulating cooling system. 0.5 M acetic acid was used at the anode and 0.5 M NaOH at the cathode. Enzyme activity was demonstrated with an agarose gel overlay technique (Spencer et al. 1964). PAGIF was carried out with a gradient of pH 4—6.5 at a gel concentration of 5% and cross linking of C = 3%. Ampholine concentration was 2.2%. Plates had the sizes of $245 \times 110 \times 1$ mm. Cooling temperature was kept at +10° C. At the anode glutamic acid in 0.5 M H_3PO_4 was used, at the cathode 0.1 M β-Alanin. Isoelectrofocusing was carried out in the Multiphor chamber at maximally 1200 V, 35 mA, and 25 W for 175 min.

Results and Discussion

Figure 1 demonstrates the PGM_1 phenotypes as observed by analysis of hemolysates by PAGIF; nine of the 10 expected phenotypes are shown. In Fig. 2, the PGM_1 phenotypes as observed by AGIF are presented. There is a good correspondence of phenotypes between PAGIF and AGIF. However, analysis of hemolysates by AGIF appeared to result in more easily classifiable PGM_1 subtypes.

In Table 1 the distribution of PGM_1 phenotypes and alleles is given for a sample of 470 unrelated individuals from Southern Germany. In this Table the

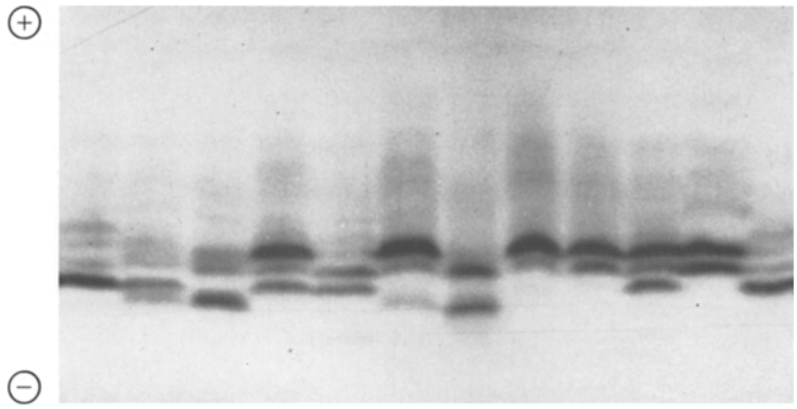

Fig. 2. PGM_1 subtypes after 90 min AGIF. From left to right: 1+1+, 1+1−, 1−1−, 1+2+, 1+2−, 1−2+, 1−2−, 2+2+, 2+2−, 1+2+, 2+2−, and 1+1+

Table 1. Distribution of phenotypes and alleles in the PGM_1 system in a sample from Southern Germany

Phenotype	Observed		Expected		Allele frequencies
	n	%	*n*	%	
PGM_1 1+1+	175	37.23	181.37	38.59	PGM_1^{1+} 0.6212
1+1−	76	16.17	71.47	15.21	PGM_1^{1-} 0.1224
1−1−	6	1.28	7.04	1.50	PGM_1^{2+} 0.2043
1+2+	127	27.02	119.30	25.38	PGM_1^{2-} 0.0521
1+2−	31	6.59	30.42	6.47	
1−2+	21	4.47	23.51	5.01	
1−2−	6	1.28	5.99	1.27	
2+2+	17	3.62	19.62	4.17	
2+2−	10	2.13	10.01	2.13	
2−2−	1	0.21	1.27	0.27	
Total	470	100.00	470.00	100.00	

$\Sigma\chi^2 = 1.8474$; $df = 6$; $P > 0.20$
Note: Phenotype PGM_1 2−2− was found later

nomenclature proposed by Bark et al. (1976) is used. Our results are in close agreement with the distribution found by Kühnl and Spielmann (1978) in Hessen, Germany.

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