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Isoelectric Focusing Studies on the PGM_1 Subtypes in the Northern Japanese Population

I. M. Sebetan, T. Kudo, K. Hiraiwa, Y. Ohno, F. Kuroda, and S. Akaishi

Dept. of Forensic Medicine, Tohoku University School of Medicine, Sendai, Japan

Summary. The distribution of the human red cell phosphoglucomutase (PGM_1) subtypes in samples from Japanese population ($n = 277$) living in the Miyagi Prefecture, the northern part of Japan, was investigated by applying the thin-layer polyacrylamide gel isoelectric focusing. In our population sample all the ten common phenotypes were demonstrated, and the estimated allele frequencies for the genes PGM_1^{1+}, PGM_1^{1-}, PGM_1^{2+}, and PGM_1^{2-} were 0.671, 0.107, 0.161, and 0.061, respectively. Family studies ($n = 40$) indicated an autosomal codominant inheritance and confirmed the four alleles. The new system will increase the probability of exclusion in paternity cases among Japanese to 29.4% compared with 14.3% if the two allele system is used.

Key words: PGM_1 polymorphism – Phosphoglucomutase-1-subtypes, rare allele

Zusammenfassung. Die Verteilung der menschlichen Erythrozyten-PGM_1-Untergruppen wurde an einer Bevölkerungsstichprobe ($n = 277$) in Miyagi, einer nördlichen Region Japans, mittels Dünnschicht-Polyacrylamidgel-isoelektrischer-Fokussierung untersucht. In unseren Stichproben wurden alle gewöhnlichen Phänotypen klar erkennbar nachgewiesen und die Allelfrequenzen berechnet. $PGM_1^{1+} = 0.671$, $PGM_1^{1-} = 0.107$, $PGM_1^{2+} = 0.161$ und $PGM_1^{2-} = 0.061$. Familienstudien ($n = 40$) zeigten den autosomal kodominanten Vererbungsmodus an und bestätigten die Existenz von vier Allelen. Das neue System erweitert die Vaterschaftsausschlußchance unter den Japanern auf 29.4% im Vergleich zu 14.3%, wenn das Zweiallelsystem benutzt wird.

Schlüsselwörter: PGM_1-Polymorphismus – Phosphoglucomutase-1-Untergruppen, seltenes Allel

Introduction

Electrophoretic separation of the human red cell phosphoglucomutase (PGM_1) polymorphism disclosed three common phenotypes, PGM_1 1, PGM_1 2-1, and

Offprint requests to: Prof. Dr. S. Akaishi (address see above)

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PGM_1 2, which are under control of two common autosomal alleles PGM_1^1 and PGM_1^2 (Spencer et al. 1964). Applying isoelectric focusing (IEF) technique to the separation of the PGM_1 phenotypes has revealed more complex band patterns than previously detected by gel electrophoresis (Ishimoto and Kuwata 1972). Bark et al. (1976) attributed such complexity to the existence of four common alleles at the PGM_1 gene locus, designated, PGM_1^{1+}, PGM_1^{1-}, PGM_1^{2+}, and PGM_1^{2-}, and the new resultant phenotypes are termed PGM_1 1+, PGM_1 1+1−, PGM_1 1−, PGM_1 2+, PGM_1 2+2−, PGM_1 2−, PGM_1 2+1+, PGM_1 2+1−, PGM_1 2−1+, and PGM_1-2−1−. Family studies (Sutton and Burgess 1978; Kühnl and Spielmann 1978) showed that there is no exception from the rule of inheritance and these new alleles are inherited according to Mendel's law.

Concerning the application of the new phenotypes in cases of disputed paternity, Kühnl and Spielmann (1978) stated that the use of IEF among Germans will increase the chance of excluding a man falsely accused of paternity from 14.5% to 25.4%, while Welch et al. (1979) applied both IEF and starch gel electrophoresis (SGE) techniques for red cell PGM_1 phenotyping in 95 cases of disputed paternity. Fifteen men were excluded from paternity on the basis of IEF compared with seven excluded by SGE.

The present investigation deals with the distribution, gene frequencies, and the mode of inheritance of the PGM_1 subtypes among the Japanese population in the northern part of Japan. The new patterns of two rare alleles were also described.

Materials and Methods

Preparation of Samples

Red Cell Hemolysate. Samples of venous blood from 277 unrelated individuals and 40 families with 55 children were drawn into test tubes and centrifuged at 3,000 rpm for 5 min. The supernatant was removed by suction, and the red cells were washed three times with 0.9% saline. Hemolysates were prepared by ultrasonic disintegration of the washed cells. Samples were stored frozen at −20° C until analysis.

Isoelectric Focusing

The run was performed with the LKB 2117 Multiphor electrofocusing equipment (Bromma, Sweden). Polyacrylamide gels of pH 5–7 and dimensions of 6.2 × 11.5 × 0.1 cm were prepared by mixing the following solutions: 2.75 ml acrylamide stock solution (29.1% (w/v) acrylamide, and 0.9% (w/v) N,N'-Methylene-bisacrylamide), 4.8 ml of distilled water in which 1.04 g sucrose was dissolved, and 0.4 ml of Ampholine (pH range 5–7 LKB). The solution was degassed under vacuum for a few minutes, then 0.15 ml of riboflavin (10 mg%, w/v) was added, and the solution was pipetted into the mould after mixing. When polymerization under fluorescent light was completed after about 2 h, the mould was stored in a refrigerator overnight before use. The samples were applied on the surface of the gel using 5 × 7 mm filter papers at a distance of 1.5 cm from the anodal electrode strip. The anodal strip was soaked with 1% aqueous solution of acetic acid, while 1% aqueous solution of ethanolamine was used for the cathodal strip. An auto-conversion power unit (Model 2000-200, KPI power supply) was used for the power source. The voltage increased gradually from 450 V to 1,200 V within 60 min, then a constant voltage of 1,200 V was applied for the duration of focusing, i.e., for about 3 h. A cooling system was used to provide efficient refrigeration for the glass supporting the gel. The circulating water during running had a temperature of +2 °C. Preservation of the gel was performed after complete removal of the over-layer agar with substrate from the surface of the gel, by immersion in tap

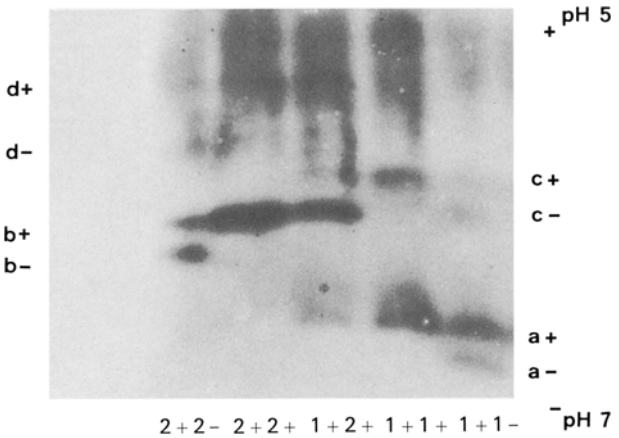

Fig. 1. IEF polyacrylamide gel showing the band patterns of five of the ten common PGM_1 phenotypes

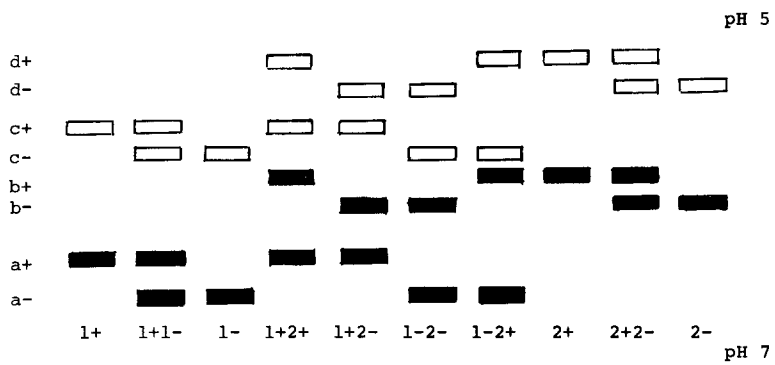

Fig. 2. Isofocusing band patterns of the common PGM_1 phenotypes

water for about 30 min. Thereafter, the gel was wrapped in a cellophane sheet and left to dry at room temperature.

Starch Gel Electrophoresis

This was carried out according to the method of Spencer et al. (1964) whose stain was also used for IEF.

Results and Discussion

Common PGM_1 Subtypes

Figure 1 shows the band patterns of five of the ten common human red cell PGM_1 polymorphism phenotypes electrofocused in pH 5–7 gradient. The common phenotypes are given in Fig. 2. The homozygous PGM_1 1 and PGM_1 2 consist of either one or two major cathodal zones with corresponding minor anodal pattern, while the heterozygous phenotypes exhibit composite patterns.

Table 1. Distribution of the PGM_1 subtypes in the northern Japanese population

Phenotypes	Number observed	Frequency observed (%)	Number expected	Frequency expected (%)
1+	128	46.1	125	45.0
1+1−	38	13.7	40	14.4
1−	3	1.1	3	1.1
1+2+	57	20.6	60	21.6
1+2−	21	7.6	23	8.2
1−2+	10	3.6	9	3.4
1−2−	5	1.8	4	1.3
2+	8	2.9	7	2.6
2+2−	6	2.2	5	2.0
2−	1	0.4	1	0.4
	277	100%	277	100%

Estimated allele frequencies: PGM_1^{1+}: 0.671 PGM_1^{2+}: 0.161
PGM_1^{1-}: 0.107 PGM_1^{2-}: 0.061
0.778 0.222

Rare variants[a]
1+7 (1.06%)
2+7 (0.035%)
1+6 (0.035%)

[a] Excluded from calculations

Distribution of the observed phenotypes is shown in Table 1. Estimated gene frequencies of our samples were: $PGM_1^{1+} = 0.671$, $PGM_1^{1-} = 0.107$, $PGM_1^{2+} = 0.161$, and $PGM_1^{2-} = 0.061$. The sums of the new gene frequencies, i.e. 1+ and 1− or 2+ and 2−, are generally quite similar to the old ones so far reported by Japanese authors (Shinoda and Matsunaga 1970a, b; Ishimoto 1970; Omoto and Harada 1970), especially those obtained in the same area (Harada et al. 1971). These findings confirm the hypothesis that the PGM_1^{1+} and PGM_1^{1-} gene products detected by IEF are represented by PGM_1^1 seen by SGE, while PGM_1^{2+} and PGM_1^{2-} gene products are represented by PGM_1^2. A good agreement was found between the observed and the expected values assuming Hardy-Weinberg equilibrium condition ($\chi_0^2 = 1.2$; $0.95 < P < 0.99$, $df = 6$).

The reported gene frequencies in some different population samples are given in Table 2, in which the gene frequencies in the present study are also given. The calculated gene frequencies of our samples are in accordance with other investigations in Japan except for those reported in Tokyo. With regard to the other populations, Europeans and Japanese are almost similar, while marked differences are observed between Japanese and the Negro population from Gambia (West Africa).

Variant PGM_1 Phenotypes

In the course of the PGM_1 phenotyping of our samples using IEF, five rare variant phenotypes were observed, i.e., 1+6(1), 1+7(3), and 2+7(1). The PGM_1 6

Table 2. Comparison of the reported gene frequencies of the PGM_1 subtypes among different populations

Population	*N*	Allele frequencies				References
		PGM_1^{1+}	PGM_1^{1-}	PGM_1^{2+}	PGM_1^{2-}	
English	123	0.6341	0.1138	0.1829	0.0691	Bark et al. (1976)
	101	0.618	0.122	0.142	0.118	Sutton and Surgess (1978)
	329	0.6367	0.1094	0.1778	0.0759	Welch et al. (1979)
German	741	0.6289	0.1397	0.1774	0.0540	Schmidtmann (1977)
	291	0.6186	0.1426	0.1718	0.0670	Kühnl et al. (1977a)
	765	0.6320	0.1242	0.1895	0.0542	Kühnl et al. (1977b)
	470	0.6212	0.1224	0.2043	0.0521	Weidinger and Schwarzfischer (1980)
West Africa (The Gambia)	637	0.7951	0.0526	0.1334	0.0189	Welch et al. (1978)
Japanese						
Tokyo	156	0.6154	0.0833	0.2340	0.0673	Maneyama et al. (1978)
Mie Pref.	852	0.697	0.090	0.157	0.056	Kuwata and Ishimoto (1978)
Osaka	388	0.6907	0.0889	0.1662	0.0451	Toyomasu and Matsumoto (1980)[a]
Miyagi Pref.	277	0.671	0.107	0.161	0.061	This study

[a] $PGM_1^{var.}$: 0.0090

major isozyme band pattern was more cathodal than the 1− zone, while the PGM_1 7 isozyme band pattern was more anodal than the 2+ zone, which exactly adheres to the C+ band. The staining intensity of the variants was less than the common ones. The variants were also accompanied by minor anodal zones as seen in Fig. 3. The band patterns of both variants were easily and clearly distinguishable from the major common four isozyme band patterns using IEF. Accordingly, screening of the samples by SGE prior to IEF, as proposed by Maneyama et al. (1978) on the assumption that PGM_1 2+ and PGM_1 7 cannot be differentiated, is most probably unnecessary. On the contrary, in our experience the PGM_1 7 was more easily distinguishable by IEF than by SGE.

Family Studies

Forty families with 55 children were studied. The data obtained are presented in Table 3. Segregation of the offspring phenotypes of children in different family combinations showed complete agreement between observed and predicted ones, approving the hypothesis of a genetic model consisting of four common alleles and ten phenotypes.

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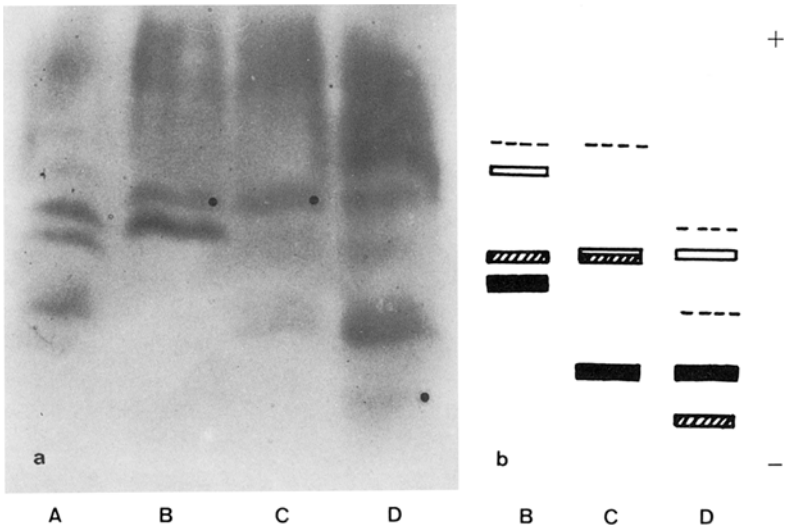

Fig. 3. a IEF band patterns of three variant PGM_1 phenotypes. The main band is marked with a dot. *A*, mixture for control; *B*, 2+7; *C*, 1+7; and *D*, 1+6. **b** Diagram of these phenotypes

Table 3. Segregation of the PGM_1 subtypes in different family combinations

Phenotypes of parents	Number of families	Number of children	Possible phenotypes of offspring	Observed phenotypes of offspring
1+1+ × 1+1+	5	8	1+1+	1+1+(8)
1+1+ × 1+1−	8	12	1+1+, 1+1−	1+1+(4), 1+1−(8)
1+1+ × 1+2+	10	13	1+1+, 1+2+	1+1+(9), 1+2+(4)
1+1+ × 1+2−	4	6	1+1+, 1+2−	1+1+(3), 1+2−(3)
1+1+ × 1−2+	2	2	1+1−, 1+2+	1+1−(2)
1+1+ × 1−2−	1	1	1+1−, 1+2−	1+1−(1)
1+1− × 1+2+	3	4	1+1+, 1+1−, 1+2+, 1−2+	1+1+(2), 1+1−(1), 1+2+(1)
1+2+ × 1+2+	2	2	1+1+, 1+2+, 2+2+	1+2+(1), 2+2+(1)
1+2+ × 1−2+	1	3	1+1−, 1+2+, 1−2+, 2+2+	1+2+(1), 1−2+(1), 2+2+(1)
1+2+ × 2+2−	2	2	1+2+, 1+2−, 2+2+, 2+2−	2+2+(1), 2+2−(1)
1+1+ × 1+7	1	1	1+1+, 1+7	1+7 (1)
2+2+ × 1+7	1	1	1+2+, 2+7	2+7 (1)
Total	40	55		

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