ELECTROPHORETIC VARIANTS OF BLOOD PROTEINS IN JAPANESE

I. PHOSPHOGLUCOMUTASE-2 (PGM2)

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Summary   A total of 17,126 individuals, of whom 11,833 are unrelated, living in Hiroshima and Nagasaki were examined for erythrocyte phosphoglucomutase-2 (PGM2) by starch gel electrophoresis using TEMM buffer, pH 7.4.   Four kinds of hereditary rare variants were encountered, three detected in single families and the one remaining in 9 unrelated families.   In addition, a fresh mutant whose main band migrated slightly cathodal to the *d*-band was detected in a male child in Nagasaki, whose parents were proximally exposed to the atomic bomb in that city.   The results described here confirm our previous data that PGM2 variation is quite low among the Japanese.

INTRODUCTION

Studies to detect mutations at the protein level to evaluate genetic effects of the atomic bombs were initiated some 10 years ago at RERF (Radiation Effects Research Foundation, Hiroshima and Nagasaki).   After a pilot study of A-bomb survivors, an electrophoretic study of children of survivors was undertaken, and interim reports were published (Neel *et al.*, 1980; Satoh *et al.*, 1982a, 1982b).   By

the end of September 1982, a total of 11,534 children of proximally exposed parents and 9,092 children of distally exposed parents were examined for variants in a maximum of 30 protein systems, the number of systems examined differing from child to child. Using the same procedure as that of Harris et al. (1974) and Neel et al. (1980), the number of equivalent locus tests was calculated as 543,664 for the exposed group and 386,706 for the control group. Since three and two fresh mutants were encountered respectively in the exposed group and the control group, giving mutation rates of 0.55×10^{-5} and 0.52×10^{-5} per locus per generation, it is clear that so far no measurable genetic effect due to A-bomb exposure of the parents has been observed. A study to detect mutations using decreased enzyme activity as a marker was added to the study in 1979, but so far no mutations have been detected in 49,076 equivalent locus tests of the combined groups (Satoh et al., 1983).

The present series of reports will describe the combined results of the electrophoretic studies on A-bomb survivors and their offspring, as a contribution towards delineating the frequency and types of variation of proteins in the Japanese population. This paper describes the results of our study of phosphoglucomutase-2 (PGM2).

SAMPLES AND FAMILY STUDIES

The subjects of most of the reports in this series are members of two studies which are being conducted at RERF, i.e., the 'Adult Health Study (AHS)' and the 'Investigation of the effects of radiation upon protein structure in children of A-bomb survivors (F_1-Biochemical Genetics Study, F_1-BGS).' The AHS is a program of medical surveillance of approximately 20,000 individuals (original number), comprising a group exposed to the atomic bombs in Hiroshima and Nagasaki, and a sex and age matched non-exposed control group. Although, for their biennial clinical examination, AHS subjects are selected at random (except for A-bomb exposure status), for this study, the parents of children who are subjects of the ABCC-RERF genetic studies (Neel and Schull, 1956; Neel et al., 1974) were given first priority in the selection. Our group of 4,649 AHS subjects examined electrophoretically is designated as the 'Adult' population. Since all the subjects in this population, 3,066 from Hiroshima and 1,583 from Nagasaki, were born before the bombing, their protein structure would not have been affected by radiation exposure, and therefore the data obtained from exposed and non-exposed individuals were combined. Inasmuch as results of earlier electrophoretic studies performed on 4,029 samples from the Adult population have already been reported (Ferrell et al., 1977; Ueda et al., 1977; Satoh et al., 1977; Tanis et al., 1978; Neel et al., 1978), in this and subsequent papers description of the characteristics of rare variants is restricted to those encountered in 620 individuals who were examined after the five reports cited above were published; however, the number of each of variant is counted on the basis of the entire number of individuals examined, which differs from system

to system.

Members of F_1-BGS population constitute a part of the 'F_1 Mortality Study' comprising two groups of children: children of proximally exposed survivors of the atomic bombs in Hiroshima and Nagasaki (either or both parents were exposed within 2,000 m from the hypocenter) and children of distally exposed survivors selected as controls for the first group, matched by sex and age (either or both parents were exposed beyond 2,500 m from the hypocenter). Since no measurable genetic effect related to the exposure experience of the parents was observed as described in the INTRODUCTION, data obtained from the two groups were pooled, and the population is designated as 'Child.' About 30% of its members are siblings and some parents of its members are included in the Adult population. In order to exclude bias where relationship between the examinees might be known, all laboratory tests were performed on samples labeled with an I.D. number only.

Since kinship is involved among some individuals in the Adult and the Child populations and within the latter population, results are reported here and in subsequent papers as follows: those dealing primarily with rare variants found in the respective populations are presented as they are; next, data from both populations are pooled and grouped by families, and a single individual is selected from each family to define a population of unrelated individuals designated as 'Representative,' from which the frequency of alleles are calculated. Inasmuch as data from 4,029 subjects in the Adult population have already been published, priority was given to parents in selecting members for the Representative population. Next, children with no siblings were selected from among those whose parents are not in the Adult population, while for children with siblings, the first among the siblings to undergo the test was selected. In the event a number of siblings underwent the test on the same day, the first one to receive the test was selected as the representative of the family.

Family study was conducted to ascertain whether or not a variant was of a genetic nature. As the purpose differed between the two studies, the method of the family study also differed in the two populations. For the Adult population, family study was pursued until a variant identical to that of the propositus was encountered (Ferrell et al., 1977), while in the case of the Child population, the purpose was specifically to examine parents (Neel et al., 1980). In either population, while some families were cooperative, others were not or family members did not reside in Hiroshima or Nagasaki. In some cases, family studies were completed automatically because the parents were included in the Adult population, and the offspring in the Child population. However, even when those who underwent family study as parents of offspring in the Child population happened to also be in the AHS population, the results of the examination were not included in the count for the Adult population. On the other hand, when those who were examined as family members of the Adult population also happened to be included in the Child population, the data were incorporated into those for the Child popu-

lation, but were excluded when frequencies were calculated since the family representative was the parents.

MATERIALS AND METHODS

Sample preparation and storage. Ammonium-potassium oxalate was used as anticoagulant for most of the blood samples obtained from the members of the Adult and some of the members of the Child, but most samples obtained from the latter was drawn into ACD solution (Formula A of Beutler, 1975). The Hiroshima blood samples were stored at 4°C for 1–3 days and separated into plasma and red cells by centrifugation at 1,200 × *g* for 20 min. Washing of the red cells and preparation of hemolysates (1 : 1) were performed as described previously (Ferrell *et al.*, 1977; Ueda *et al.*, 1977). Aliquots of plasma and the washed packed red cells were stored at −70°C and in liquid nitrogen and processed when needed. Most of the samples obtained in Nagasaki were immediately separated into plasma and erythrocytes, the plasma and the washed erythrocyte layer were frozen at −70°C and transported to Hiroshima on dry ice. When the study of enzyme activity measurement commenced in 1979, whole blood samples obtained in Nagasaki were kept for 1–3 days at 4°C, sent to Hiroshima on ice, and then processed as described above for the Hiroshima samples. Although conditions of transportation and preservation of the samples were not always the same during the period of screening, careful review showed that the electrophoretic results were not affected. Samples stored in liquid nitrogen were first used for repeat tests and comparison between variants, but when possible, freshly drawn blood was used. Even bands of a low activity variant could be clearly detected using samples stored in liquid nitrogen for 7–8 years, and the mobility of the variant band in those samples was not different from that in fresh samples for 30 protein systems.

Vertical starch gel electrophoresis. Electrophoresis was conducted at 4°C and 7 volts/cm for 20–23 hr employing Tris-EDTA-maleic acid-$MgCl_2$ (TEMM) buffer, pH 7.4 of Spencer *et al.* (1964), as bridge buffer and its 1/15 diluted solution (pH 7.4) as gel buffer. For the first screening, 13.3% concentration of Electrostarch (Electrostarch Co., Madison, Wisconsin, U.S.A.) gels were mainly used but some comparison runs of variants were also carried out on Connaught starch gels (13.3%). Staining by phosphoglucomutase (PGM) activity was carried out according to the method of Spencer *et al.* (1964). However, 6,928 samples from children were stained directly by applying a staining solution to the gel surface with a brush instead of by agar overlay. Staining on the basis of phosphopentomutase (PPM) activity was performed using the labile phosphate detection method of Quick *et al.* (1972), with ribose-5-phosphate as the substrate. For PPM reaction, the gel was immersed in the reaction solution instead of by agar overlay, and incubated at 37°C for 1 hr.

PGM activity measurement. Methods employed for determination of the PGM

activity were the same as those described in a separate paper concerning a low activity variant of PGM1 (Satoh et al. in preparation) which are in principle based on the methods recommended by Beutler (1975) and ICSH (Beutler et al., 1977). Mean ± standard deviation (SD) of PGM activity for Hiroshima samples (n = 191) and Nagasaki samples (n = 195) were 1.82 ± 0.23 IU/gHb (international unit per gram of hemoglobin) and 1.85 ± 0.19 IU/gHb, respectively, whose PGM1 and PGM2 phenotypes were both 1. PGM activity was determined whenever possible for propositi having rare variants, and electrophoresis and determination of PGM activity were also performed for their families. The activity of each variant will also be referred to when describing their characteristics.

Nomenclature of rare variants. Rare variants were named according to the method of Ferrell et al. (1977) except that abbreviations for Hiroshima and Nagasaki are HR and NG, respectively.

RESULTS

In the previous paper (Satoh et al., 1977), we scored the data obtained by electrophoresis using both a TEMM buffer and a histidine-citrate discontinuous buffer system (Fildes and Harris, 1966), but here only the determinations with TEMM buffer will be described, since we found that some slow moving variants of PGM2 may be missed in the histidine-citrate discontinuous buffer system. Thus, the number of examination for PGM2 is less than the number of subjects who participated in the study for both Adult and Child populations.
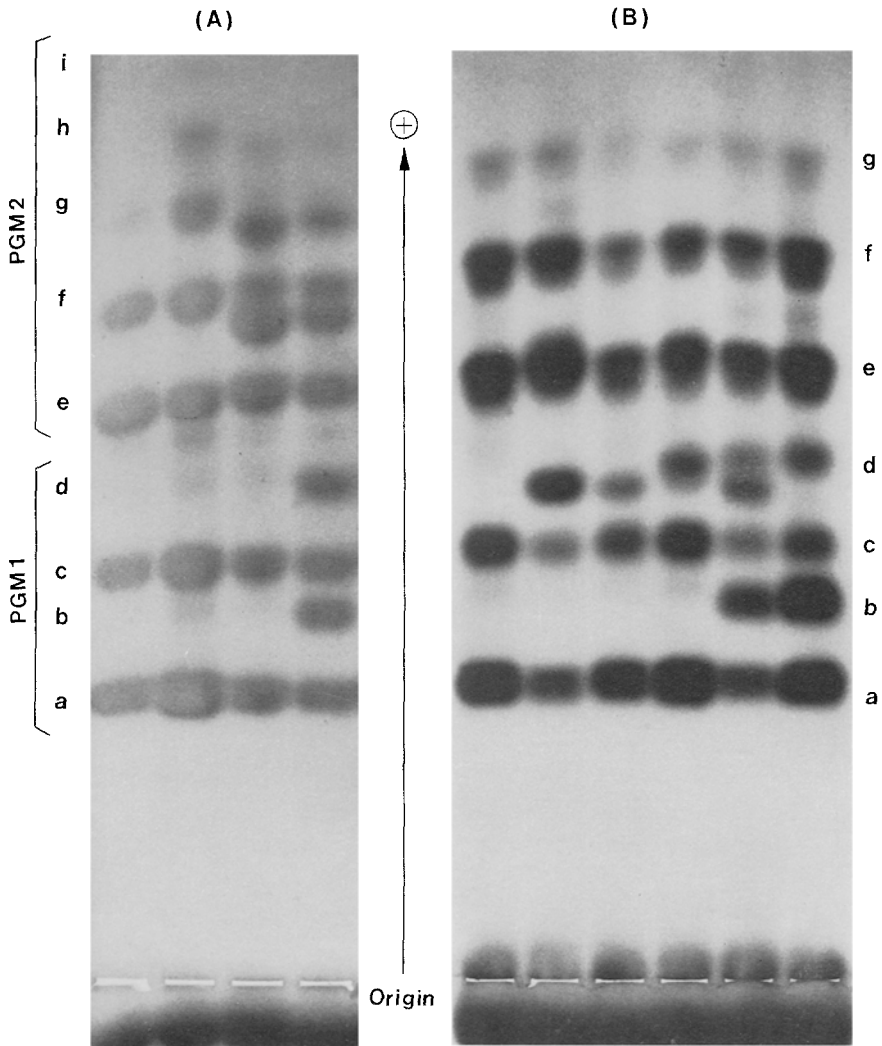
Common variants present in polymorphic frequency were not observed in examinations of 2,534 subjects in the Adult population and 14,592 in the Child population.

One type of rare variant was detected in the Adult population and five variants one of which is identical to that in the Adult population, were encountered in the Child population. Figure 1 is a diagram of these PGM2 variants and Fig. 2 shows their electrophoretic patterns on the starch gel. All showed phosphopentomutase (PPM) activity confirming them as allozymes of PGM2 (Fig. 3).

In Table 1, numbers of individuals with various phenotypes in each population are shown. When the phenotype could not be read clearly, 'no type' is indicated. Representatives with 'no type' were excluded in selecting the Representative population. Those cases aside, the representatives were selected by the method described in SAMPLES AND FAMILY STUDIES. Sometimes, therefore, when there was a member with a normal type and a member with a variant in the family, the former was selected and the latter excluded from the Representative population. Results of family studies are shown in Table 2.

PGM2 2_{HR1}
In a female Hiroshima child with I.D. No. 958460, 5 bands of PGM2 were

Fig. 1. Diagram of PGM2 variants on starch gel employing TEMM buffer, pH 7.4 (Gel buffer is 1/15 dilution of bridge buffer).

detected: along with *e-*, *f-*, and *g*-bands, *h-* and *i*-bands were observed anodally to these three bands. The order of intensity of these bands for PGM and PPM activity was $f=e\geqq g>h>i$. Mobility of these bands was very similar to that of the five allozyme bands of PGM2 1-2 detected by Hopkinson and Harris (1966) in Negroes living in England and Africa. According to our rules of nomenclature, this variant (No. 1 in Table 2) was designated PGM2 2_{HR1} and was confirmed to be a genetic variant by the family study. Mother, uncle (mother's younger brother) and a younger brother of the propositus showed the same variant as that of propositus, while another brother, another uncle (mother's younger brother) and an aunt (elder sister of mother) were PGM2 1.

PGM2 5_{NG1} and PGM2 5_{NG2}

Two fast variants (No. 2 and No. 3 in Table 2) whose mobilities were almost

(A) **(B)**

Fig. 2. Five types of PGM2 variants migrating faster (A) and slower (B) than PGM2 1
on Electrostarch gel, stained for PGM activity.

identical each other on starch gel were observed in two female Nagasaki children
who are not related. Mobility and intensity of variant bands stained on the basis
of PGM activity were similar to those of PGM2 1-5 in a diagram presented by
Parrington *et al.* (1968). Variant bands moved so close to *f*- and *g*-bands that they
were almost in contact with cathodal side of *f*- and *g*-bands and the third band
moved to the anodal side of *g*-band. The intensities of these three bands were

PGM 2 | 1 1-2$_{HR1}$1-5$_{NG1}$1-5$_{NG2}$1-9$_{NG2}$1-9$_{NG1}$1-9$_{NG1}$1
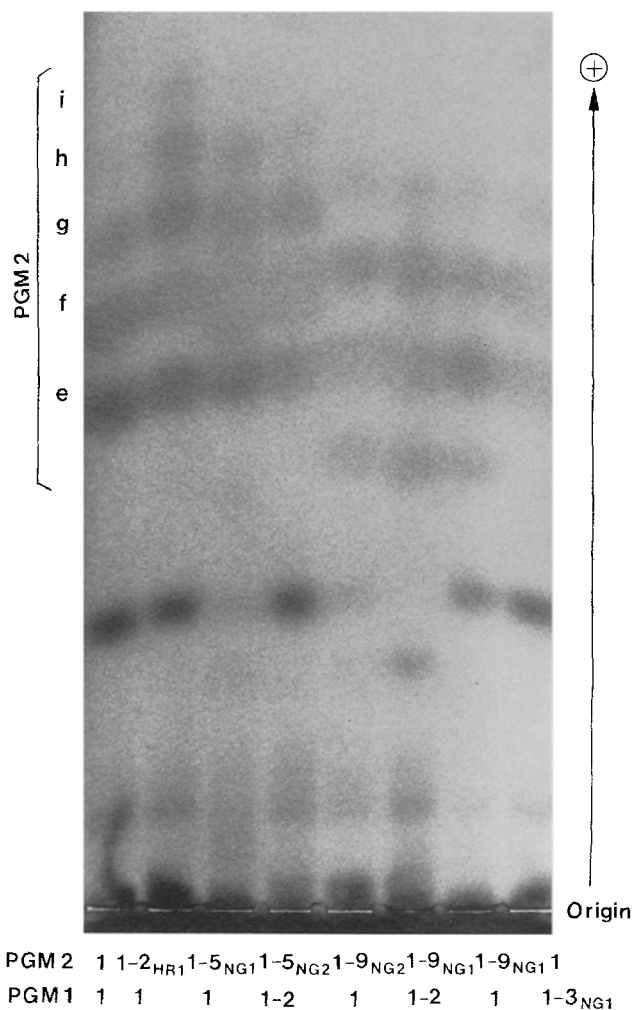PGM 1 | 1 1 | 1 | 1-2 | 1 | 1-2 | 1 | 1-3$_{NG1}$

Fig. 3. Five types of PGM2 variants stained for phosphopentomutase activity, encountered in the Hiroshima and Nagasaki populations.

similar respectively to those of heterozygous *e-, f-* and *g*-bands of these same individuals. Electrophoresis of the hemolysates from these 2 children was repeated several times together on the same starch gel using TEMM buffer and only when the separation of the bands was excellent, was a subtle difference in mobility between these variants observed. On the other hand, on polyacrylamide thin layer gel isoelectric focusing, isoelectric point (pI) of the major band of the variant No. 2 which moved slower on the starch gel was 5.9 and that of the variant No. 3 was 5.8. In this examination, pI for the *e*-band was 6.0. The former variant was designated PGM2 5$_{NG1}$ and the latter PGM2 5$_{NG2}$ (Takahashi and Satoh, 1982). In

Table 1. Various phenotypes of PGM2 encountered among Japanese of two populations and the Representative population.[a]

Phenotype	Population				
	Adult	Child	Representative [a]		
			Combined	Hiroshima	Nagasaki
1	2,529	14,577	11,825	6,673	5,152
1-2HR1	0	1	1	1	0
1-5NG1	0	1	0	0	0
1-5NG2	0	1	1	0	1
1-9NG1	3	7	6	4	2
1-9NG2	0	1	0	0	0
No type[b]	2	4	0	0	0
Total	2,534	14,592	11,833	6,678	5,155

[a] Representative population is composed of the unrelated individuals selected from the Adult and the Child populations. Variants found in the latter two populations are not necessarily encountered in the Representative population. See text for definition of this population. [b] The phenotype could not be read clearly.

Table 2. Summary of the results of family studies of variants of PGM2 encountered in the population of the Child.

Propositus					Mother	Father	Other family member	Comments
Variant No.	City	Variant type	ID No.	Sex				
1	H	2HR1	958460	F	♀	♂		*1
2	N	5NG1	706056	F	♀	♂	♂ Brother	pI = 5.9
3	N	5NG2	728309	F	♀	♂		pI = 5.8
4	N	9NG1	158952	F ⌉	♀	Dead	♂ Brother	
5	N	9NG1	722344	M ⌋				
6	H	9NG1	249460	M	♀	♂		
7	H	9NG1	275296	M	♀	♂	⌈ ♀ Sister	
8	H	9NG1	303594	M	♀	♂	⌊ ♂ Brother	
9	N	9NG1	729430	M	♀	♂		
10	H	9NG1	930206	M	♀	♂	⌈ ♂ Brother	
11	N	9NG2	714546	M	♀	♂	⌊ ♀ Sister	Mutation

♀,♂ Heterozygote for variant alleles at *PGM2* locus
♀,♂ Homozygote for normal *PGM2*1*
][Siblings
*1 For results of examination on other family members, see text

family studies, mother of the propositus showing PGM2 1-5$_{NG1}$ showed the same variant and father and a brother showed PGM2 1: father of the propositus showing PGM2 1-5$_{NG2}$ had the same variant as his daughter and mother was PGM2 1.

PGM2 9$_{NG1}$

In addition to three allozyme bands of PGM2, *e, f, g*, a band with slow mobility and weak intensity was detected in two Hiroshima individuals and one Nagasaki individual among 2,532 clearly typed (1,299 in Hiroshima and 1,233 in Nagasaki) in the Adult population. The band moved to the equal position of the major band of PGM1 3$_{NG1}$ (Satoh *et al.*, 1977) and slightly more cathodally than *d*-band of PGM1 on the Electrostarch gel, though on the Connaught starch gel, it sometimes moved slightly more cathodally than the major band of PGM1 3$_{NG1}$ depending on starch lot. No minor band was observed either on the Electrostarch gel or on the Connaught starch gel. One of these variants was included in the population described in a previous report, but it had been named PGM1 3$_{NG2}$ because its mobility was similar to that of PGM1 3$_{NG1}$ (Satoh *et al.*, 1977), though the band intensity was weak. Following a suggestion by Dr. Hopkinson that the variant might be PGM2 instead of PGM1, PPM activity was determined and found to be positive, confirming this surmise. It was also observed that the intensities of *e-, f-* and *g-* bands of this individual were weaker than that of the normal type for both PGM activity and PPM activity suggesting heterozygosity. This variant was renamed PGM2 9$_{NG1}$ because its mobility is similar to that of PGM2 9 detected in Papua-New Guinea by Blake and Omoto (1975). Therefore, the phenotype of the foregoing three cases is PGM2 1-9$_{NG1}$. Intensity of the band of PGM2 9$_{NG1}$ was weaker than those of *e-* and *f*-bands and stronger than that of *g*-band for both PGM activity and PPM activity. Intensity of the band of 9$_{NG1}$ by PGM activity was markedly weaker than that of homozygous *c*-band.

PGM2 1-9$_{NG1}$ was also detected in four Hiroshima and three Nagasaki children (variant Nos. 4 to 10) among 14,588 clearly typed children (7,594 in Hiroshima and 6,994 in Nagasaki). Two of the three Nagasaki children were siblings (variant Nos. 4 and 5). Among 10 individuals in the two populations for whom PGM2 1-9$_{NG1}$ was detected, phenotype of PGM1 was 1 in eight cases and 1-2 in two cases. In the latter case, *i.e.*, samples which show PGM1 1-2 and PGM2 1-9$_{NG1}$, the band of PGM2 9$_{NG1}$ clearly separates near *d*-band between *c*-band and *d*-band under excellent electrophoretic conditions (Figs. 1 and 2), but when good separation could not be obtained due to a slight difference of starch lot or electrophoretic conditions, little difference could be observed compared with PGM1 1-2 and PGM2 1 except that the *d*-band was wider than normal and its intensity was similar to or stronger than the *b*-band. The frequency of *PGM2*9NG1* allele in the Representative population was 0.00025.

Family studies for one propositus from Nagasaki in the Adult population could not be done. One son of each of the other two propositi from Hiroshima in the Adult population had the same variant as the mother. Among seven children of

the Child population, for five propositi, both parents were examined and PGM2 9_{NG1} was encountered in one or the other parent (Table 2) confirming hereditary transmission of the variant. The other 2 children were siblings whose mother had PGM2 1 and father was deceased.

Although the intensity of allozyme band of PGM2 9_{NG1} was weak, PGM activity in eight among 10 subjects in whom PGM2 9_{NG1} was detected showed a mean value of 1.69 IU/gHb (SD=0.21 IU/gHb) or 92% of the normal type (PGM1 1 and PGM2 1) which is considered to be within normal limits.

PGM2 9_{NG2}

A variant band with mobility similar to that of PGM2 9_{NG1} was detected in a male child born in 1960 whose parents were proximally exposed in Nagasaki. Designated PGM2 9_{NG2}, the variant band was equal or slightly cathodal to the d-band, but moved slightly anodal to the band of PGM2 9_{NG1} from which it was thus distinguishable. Mobilities of the bands of PGM2 9_{NG1}, PGM2 9_{NG2}, PGM1 3_{NG1} and d-isozyme depended on type and lot number of the starch used to prepare the gels. The order of their mobilities to the anodal side is: $d \geqslant PGM2\ 9_{NG2} > PGM1\ 3_{NG1} \geqslant PGM2\ 9_{NG1}$. However, on thin layer polyacrylamide gel isoelectric focusing, PGM2 9_{NG1} and PGM2 9_{NG2} focused to the cathodal side of the main band of PGM2 1, while PGM1 3_{NG1} focused to the anodal side, approximate pIs of PGM2 9_{NG1}, PGM2 9_{NG2}, PGM2 1, PGM1 $3_{NG1}-$ and PGM1 $3_{NG1}+$ being 6.5, 6.2, 6.0, 5.9 and 5.8, respectively (Takahashi *et al.*, 1982), and, therefore, each isozyme is clearly distinguishable. The intensity of PGM activity of the band of this variant was slightly weaker than that of the homozygous c-band and obviously stronger than that of the band of PGM2 9_{NG1}. PGM and PPM activities of the e-, f-, and g-bands of the propositus were weaker than those of normal PGM2 1. Hence, the propositus' phenotype was PGM1 1 and PGM2 1-9_{NG2}. In the PGM2 1-9_{NG2}, the intensity of variant band was slightly weaker than that of e-band and stronger than that of f-band. PGM activity of the propositus was 1.79 IU/gHb which is normal.

No variant bands were detected in any of the propositus' immediate family: both parents and a younger brother and younger sister all showed PGM1 1 and PGM2 1. Blood types, protein types of the family members are shown in Table 3. Propositus and parents were examined repeatedly for blood types and protein types including PGM2 using freshly drawn blood specimens twice in six months, and the same results were obtained. HLA A, B, C typing was performed using specimens obtained at the second instance. There was no discrepancy in the data between the legal and biological parentage. Routine, C and Q band chromosome examinations conducted at the same time produced no contradictory evidence suggesting other than true biological parentage. Therefore, the variant detected in the propositus is considered to be a fresh mutant attributable to mutation occurring in one of the parents. Air dose of the mother was 226 rad for γ-ray and 2 rad for

Table 3. Blood, protein and HLA types of the parents and the propositus
having a putative mutant of PGM2 (PGM2 9_{NG2}).

	Propositus	Mother	Father	Brother	Sister
Blood types					
ABO	A_1	A_1B	A_1	A_1B	A_1B
Rh	R_1R_2	R_1R_1	R_1R_2	R_1R_1	R_1R_2
MNSs	Ns	MNs	Ns	Ns	Ns
Duffy	Fy(a+)	Fy(a+)	Fy(a+)		
Kell	K−k+	K−k+	K−k+		
Protein types					
PI (a_1-AT)	1-2	1	2-3	1-3	1-2
HP	1-2	1-2	1-2	1	1
ACP1	AB	B	AB	AB	B
ADA	1	1	1	1	1
ESD	1-2	1-2	1-2	1	2
GOT1	1	1	1	1	1
GPT	1-2	1	2	1-2	1-2
6PGD	A	A	A	A	A
PGM1	1	1	1	1	1
PGM2	$1-9_{NG2}$	1	1	1	1
PGM3	1-2	1	2	1-2	1-2
HLA-types	A9, B40, Cw3	A9, B40, Cw3	A9, B27, Cw1		
	B5	A10, B12	B5		

CDe, R_1; cDE, R_2.

Abbreviations: PI, protease inhibitor, a_1-antitrypsin; HP, haptoglobin; ACP1, acid phosphatase-1;
ADA, adenosine deaminase; ESD, esterase D; GOT1, cytoplasmic glutamate-oxaloacetate trans-
aminase; GPT, glutamate-pyruvate transaminase; 6PGD, 6-phosphogluconate dehydrogenase;
PGM1, PGM2, PGM3, phosphoglucomutase-1, -2, -3; HLA, human lymphocyte antigen.

neutron while that of the father was 10 rad for γ-ray (T65D, Milton and Shohoji,
1968).   According to calculation method of Kerr *et al.* (1979), gonadal dose to the
mother was 91.6 rad γ-ray and 0.24 rad neutron and that of the father was 6.5 rad
γ-ray.   Hematological data of the propositus are normal and he has been healthy.

DISCUSSION

In the face of the well-known diversity of PGM1 (Spencer *et al.*, 1964; Hop-
kinson and Harris, 1966; Blake and Omoto, 1975; Satoh *et al.*, 1977; Bark *et al.*,
1976; Kühnl *et al.*, 1977; Bissbort *et al.*, 1978; Scozzari *et al.*, 1981) PGM2 is mon-
omorphic in Caucasoid and most of Mongoloid populations (Hopkinson and Har-
ris, 1969), though it is polymorphic in Negroid (Hopkinson and Harris, 1966) and

certain populations such as Trio Indians (Geerdink *et al.*, 1974), Aboriginals from Central Australia (Kirk *et al.*, 1971) and the inhabitants of New Guinea (Blake and Omoto, 1975). The reported number of rare PGM2 variants is small and frequencies are low in the three human races. The difference in two enzymes is especially large in Japanese. In a previous paper, Satoh *et al.* (1977) reported that in Japanese, PGM1 is a diversified enzyme, both in type and in the frequency of variants, in contrast to PGM2 which is monomorphic and no variants were observed, despite their possible common origin through gene duplication (Hopkinson and Harris, 1969). The same phenomenon is again observed in this study in which the population is approximately 6 times larger than the previous population. In a total of 17,126 individuals of whom 11,833 are unrelated, five kinds of rare PGM2 variants were encountered in 14 individuals while 13 types of rare PGM1 variants were detected in 103 individuals (Satoh *et al.*, 1984). In Ishimoto's review (1978), a combined sample of 10,851 Japanese excluding our populations were examined for PGM1 and PGM2 and no rare variants of PGM2 were reported. Except for two PGM2 variants reported in our previous interim report (Asakawa *et al.*, 1978) which are named PGM2 5_{NG1} and PGM2 9_{NG1} in this paper, no other PGM2 variants have been described until recently Nishigaki *et al.* (1982) detected a variant similar to phenotype PGM2 1-5 of Parrington *et al.* (1968), or possibly either PGM2 1-5_{NG1} or PGM2 1-5_{NG2} found in our population, though no direct comparative studies have been made.

When variation in these two enzymes of Japanese were compared with that of an English population (Harris *et al.*, 1974), though these two island countries are similar with respect to climate, geographical position to the continent, history of populations, and grade of industrialization all of which affect the structure of population, variation in PGM1 was much higher in Japanese (Neel *et al.*, 1978). A higher mutation rate in Japanese was suggested as a possible explanation for the difference between two populations with respect to PGM1 variation.

Recently, we reported that the conventional allele *PGM1*7* which is present in polymorphic proportions in the Japanese (Satoh *et al.*, 1977), can be subtyped into *PGM1*7+* and *PGM1*7−* (Takahashi *et al.*, 1980). *PGM1*3NG1*, which is the most frequent rare variant *PGM1* allele in Japanese of Hiroshima and Nagasaki (Satoh *et al.*, 1984), can also be subtyped into *PGM1*3NG1+* and *PGM1*3NG1−* by isoelectric focusing and family studies confirmed these isoelectric point subtypes as real alleles. Considering the distribution of the conventional alleles of *PGM1*7* and *PGM1*3* in the Pacific area, and the isoelectric points of all of 8 'isoelectric point alleles,' four of which are common to all the populations of three races and four new alleles found in Japanese, we proposed an evolutionary phylogeny of 'isoelectric point alleles' of PGM1 (Takahashi *et al.*, 1982): an extension of a hypothesis originally proposed by Carter *et al.* (1979) that 'mutation and intragenic crossing over developed a series of isoelectric point alleles.' This hypothesis of gene mutation and successive intragenic crossing over seems to explain very well

the high diversity of PGM1 in the Japanese.   Since the possibility of crossing over occurring is much higher than that of the true mutation and increases with the number of alleles, 'apparent'' mutation which is a combination of the true mutation and crossing over, will increase at a much higher rate in populations in which the number of alleles is larger.   In Japanese, the presence of the *PGM1*7* allele in polymorphic proportion would appear to work for the diversity of PGM1.

In the course of examination of 1,000 individuals by IEF, we were unable to detect any isoelectric point variants of PGM2 (Takahashi *et al.*, 1982) nor have other authors (Sutton, 1979; Tipler *et al.*, 1982) described such variants of PGM2. Moreover no heterogeneity in sensitivity to heat denaturation of PGM2 isozymes was described by Scozzari (1981).   According to our hypothesis, the apparent mutation rate of PGM2 either in Japanese or Caucasoid populations will be low since there is no second allele occurring in polymorphic frequency so that intragenic crossing over may occur.

REFERENCES

Asakawa, J., Fujita, M., Goriki, K., Kageoka, T., Neriishi, S., Kawamoto, S., Hamilton, H.B., Satoh, C., and Ueda, N. 1978.   Rare electrophoretic variants found in Hiroshima and Nagasaki residents.   Proc. 23rd Annual Meeting of Jpn. Soc. Human Genet., 1978. *Jpn. J. Human Genet.* **24**: 215–216.

Bark, J.E., Harris, M.J., and Firth, M. 1976.   Typing of the common phosphoglucomutase variants using isoelectric focusing—A new interpretation of the phosphoglucomutase system.   *J. Forens. Sci. Soc.* **16**: 115–120.

Beutler, E. 1975.   *Red Cell Metabolism, A Manual of Biochemical Methods.*, 2nd ed., Grune & Stratton, New York, San Francisco, and London.

Beutler, E., Blume, K.G., Kaplan, J.C., Löhr, G.W., Ramot, B., and Valentine, W.N. 1977.   International committee for standardization in hematology: recommended methods for red-cell enzyme analysis.   *Br. J. Haematol.* **35**: 331–340.

Bissbort, S., Ritter, H., and Kömpf, J. 1978. PGM_1 subtyping by means of acid starch gel electrophoresis.   *Hum. Genet.* **45**: 175–177.

Blake, N.M and Omoto, K. 1975.   Phosphoglucomutase types in the Asian-Pacific area: a critical review including new phenotypes.   *Ann. Hum. Genet.* **38**: 251–273.

Carter, N.D., West, C.M., Emes, E., Parkin, B., and Marshall, W.H. 1979.   Phosphoglucomutase polymorphism detected by isoelectric focusing: gene frequencies, evolution and linkage.   *Ann. Hum. Biol.* **6**: 221–230.

Ferrell, R.E., Ueda, N., Satoh, C., Tanis, R.J., Neel, J.V., Hamilton, H.B., Inamizu, T., and Baba, K. 1977.   The frequency in Japanese of genetic variants of 22 proteins.   I. Albumin, ceruloplasmin, haptoglobin, and transferrin.   *Ann. Hum. Genet.* **40**: 407–418.

Fildes, R.A. and Harris, H. 1966.   Genetically determined variation of adenylate kinase in man. *Nature, Lond.* **209**: 261–263.

Geerdink, R.A., Bartstra, H.A., and Hopkinson, D.A. 1974.   Phosphoglucomutase (PGM2) variants in Trio Indians from Surinam.   *Hum. Hered.* **24**: 40–44.

Harris, H., Hopkinson, D.A., and Robson, E.B. 1974. The incidence of rare alleles determining electrophoretic variants: data on 43 enzyme loci in man. *Ann. Hum. Genet.* **37**: 237–253.

Hopkinson, D.A. and Harris, H. 1966. Rare phosphoglucomutase phenotypes. *Ann. Hum. Genet.* **30**: 167–181.

Hopkinson, D.A. and Harris, H. 1969. Red cell acid phosphatase, phosphoglucomutase and adenylate kinase. In *Biochemical Methods in Red Cell Genetics*, J.J. Unis, ed., Academic Press, New York, London, pp. 368.

Ishmioto, G. 1975. Red cell enzymes. In *Human Adaptability: Anthropological and Genetic Studies on the Japanese*, Vol. 2, S. Watanabe, S. Kondo, and E. Matsunaga, eds., Univ. of Tokyo Press, Tokyo, pp. 109–139.

Kerr, G.D. 1979. Organ dose estimates for the Japanese atomic bomb survivors. *Health Phys.* **37**: 487–508.

Kirk, R.L., Blake, N.M., Moodie, P.M., and Tibbs, J. 1971. The distribution of some serum protein and enzyme groups among populations at various localities in the Nothern Territory of Australia. *Hum. Biol. Oceania* **1**: 54–76.

Kühnl, P., Schmidtmann, U., and Spielmann, W. 1977. Evidence for two additional common alleles at the PGM_1 locus. A comparison by three different techniques. *Hum. Genet.* **35**: 219–223.

Milton, R.C. and Shohoji, T. 1968. Tentative 1965 radiation dose estimation for atomic bomb survivors, Hiroshima-Nagasaki, *ABCC Technical Report 1-68*, Radiation Effects Research Foundation, Hiroshima, pp. 1–43.

Neel, J.V. and Schull, W.J. 1956. *The Effect of Exposure to the Atomic Bombs on Pregnancy Termination in Hiroshima and Nagasaki.* NAS-NRC Publ. No. 461, Washington, D.C.

Neel, J.V., Kato, H., and Schull, W.J. 1974. Mortality in children of atomic bomb survivors and controls. *Genetics* **76**: 311–326.

Neel, J.V., Ueda, N., Satoh, C., Ferrell, R.E., Tanis, R.J., and Hamilton, H.B. 1978. The frequency in Japanese of genetic variants of 22 proteins. V. Summary and comparison with data on Caucasians from the British Isles. *Ann. Hum. Genet.* **41**: 429–441.

Neel, J.V., Satoh, C., Hamilton, H.B., Otake, M., Goriki, K., Kageoka, T., Fujita, M., Neriishi, S., and Asakawa, J. 1980. Search for mutations affecting protein structure in children of atomic bomb survivors: Preliminary report. *Proc. Natl. Acad. Sci. USA* **77**: 4221–4225.

Nishigaki, I., Itoh, T., and Hasegawa, I. 1982. A rare phenotype of phosphoglucomutase-2 first detected in Mongoloids. *Hum. Genet.* **62**: 246–249.

Parrington, J.M., Cruickshank, G., Hopkinson, D.A., Robson, E.B., and Harris, H. 1968. Linkage relationships between the three phosphoglucomutase loci PGM_1, PGM_2, and PGM_3. *Ann. Hum. Genet.* **32**: 27–34.

Quick, C.B., Fisher, R.A., and Harris, H. 1972. Differentiation of the PGM_2 locus isozymes from those of PGM_1 and PGM_3 in terms of phosphopentomutase activity. *Ann. Hum. Genet.* **35**: 445–454.

Satoh, C., Ferrell, R.E., Tanis, R.J., Ueda, N., Kishimoto, S., Neel, J.V., Hamilton, H.B., and Baba, K. 1977. The frequency in Japanese of genetic variants of 22 proteins. III. Phosphoglucomutase-1, phosphoglucomutase-2, 6-phosphogluconate dehydrogenase, adenylate kinase, and adenosine deaminase. *Ann. Hum. Genet.* **41**: 169–183.

Satoh, C., Awa, A.A., Neel, J.V., Schull, W.J., Kato, H., Hamilton, H.B., Otake, M., and Goriki, K. 1982a. Genetic effects of atomic bombs. In *Human Genetics, Part A, The Unfolding Genome* (Proc. 6th Intl. Congr. of Hum. Genet., 1981, Jerusalem, B. Bonné-Tamir ed.), Alan R. Liss. Inc., New York, pp. 267–276.

Satoh, C., Goriki, K., Hamilton, H.B., and Neel, J.V. 1982b. Search for mutations affecting protein structure in children of atomic bomb survivors. *Jpn. J. Genet.* **57**: 702.

Satoh, C., Neel, J.V., Yamashita, A., Goriki, K., Fujita, M., and Hamilton, H.B. 1983. The fre-

quency among Japanese of heterozygotes for deficiency variants of 11 enzymes. *Am. J. Hum. Genet.* **35**: 656–674.

Satoh, C., Takahashi, N., Kaneko, J., Kimura, Y., Fujita, M., Asakawa, J., Kageoka, T., Goriki, K., and Hazama, R. 1984. Electrophoretic variants of blood proteins in Japanese. II. Phosphoglucomutase-1 (PGM1). *Jpn. J. Human Genet.* **29**: in press.

Scozzari, R., Trippa, G., Santachiara-Benerecetti, A.S., Terrenato, L., Iodice, C., and Benincasa, A. 1981. Further genetic heterogeneity of human red cell phosphoglucomutase-1: a non-electrophoretic polymorphims. *Ann. Hum. Genet.* **45**: 313–322.

Spencer, N., Hopkinson, D.A., and Harris, H. 1964. Phosphoglucomutase polymorphism in man. *Nature, Lond.* **204**: 742–745.

Sutton, J.G. 1979. Characterisation of the isozymes of phosphoglucomutase (PGM) determined by the first (PGM$_1$) and second (PGM$_2$) locus observed by isoelectric focusing. *Hum. Genet.* **47**: 279–290.

Takahashi, N., Nishizaki, J., and Satoh, C. 1980. Study of *PGM1^7* by isoelectric focusing. Proc. 25th Annual Meeting of Jpn. Soc. Human Genet., 1980. *Jpn. J. Human Genet.* **26**: 155–156.

Takahashi, N., Neel, J.V., Satoh, C., Nishizaki, J., and Masunari, N. 1982. A phylogeny for the principal alleles of the human phosphoglucomutase-1 locus. *Proc. Natl. Acad. Sci. USA* **79**: 6636–6640.

Takahashi, N. and Satoh, C. 1982. Isoelectric focusing of rare variants of human phosphoglucomutase-1 (PGM$_1$) and phosphoglucomutase-2 (PGM$_2$). Proc. 27th Annual Meeting of Jpn. Soc. Human Genet., 1982. *Jpn. J. Human Genet.* **28**: 187.

Tanis, R.J., Ueda, N., Satoh, C., Ferrell, R.E., Kishimoto, S., Neel, J.V., Hamilton, H.B., and Ohno, N. 1978. The frequency in Japanese of genetic variants of 22 proteins. IV. Acid phosphatase, NADP-isocitrate dehydrogenase, peptidase A, peptidase B and phosphohexose isomerase. *Ann. Hum. Genet.* **41**: 419–428.

Tipler, T.D., Dunn, D.S., and Jenkins, T. 1982. Phosphoglucomutase first locus polymorphism as revealed by isoelectric focusing in southern Africa. *Hum. Hered.* **32**: 80–93.

Ueda, N., Satoh, C., Tanis, R.J., Ferrell, R.E., Kishimoto, S., Neel, J.V., Hamilton, H.B., and Baba, K. 1977. The frequency in Japanese of genetic variants of 22 proteins. II. Carbonic anhydrase I and II, lactate dehydrogenase, malate dehydrogenase, nucleoside phosphorylase, triose phosphate isomerase, haemoglobin A and haemoglobin A$_2$. *Ann. Hum. Genet.* **41**: 43–52.