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# ELECTROPHORETIC VARIANTS OF BLOOD PROTEINS IN JAPANESE
# I. PHOSPHOGLUCOMUTASE-2 (PGM2)

## 日本人の血液蛋白質の電気泳動上の変異型
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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 Tris-EDTA-Maleic acid-$MgCl_2$ buffer, pH 7.4. Four kinds of hereditary rare variants were encountered, three detected each in a single family and the one remaining in nine 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 (Hiroshima and Nagasaki). After a pilot study of A-bomb survivors, an electrophoretic study of

## 要 約

Tris-EDTA-Maleic acid-$MgCl_2$緩衝液，pH 7.4を用いる澱粉ゲル電気泳動法で，広島・長崎に住む総計17,126人の赤血球 phosphoglucomutase-2 (PGM2) を検査した．このうち11,833人は血縁関係のない人たちであった．親譲りのまれな電気泳動上の変異型が4種類観察されたが，このうち3種類はそれぞれ1家族にだけ見られ，残る1種類は血縁関係のない9家族から見いだされた．更に，主バンドが*d*-バンドよりもわずかに陰極側の所に移動するような新しいミュータントの1例が長崎の男の子供に検出された．この男子の両親は長崎の近距離被爆者である．この報告で得られた結果は，我々が以前に得た日本人ではPGM2の変異が非常に低いというデータを確認するものである．

## 緒 言

原爆の遺伝的影響を究明するために蛋白質レベルで突然変異を検出する研究は，放影研(広島・長崎)で約10年前に開始された．原爆被爆者について試行調査

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children of survivors was undertaken, and interim reports were published.[1-3] 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[4] and Neel et al,[1] 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 $0.55 \times 10^{-5}$ and $0.52 \times 10^{-5}$ 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.[5]

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 PGM2.

## MATERIALS AND METHODS

**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 A-bombs in Hiroshima and Nagasaki, and a sex- and age-matched nonexposed 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[6,7] 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

を行った後，被爆者の子供に対する電気泳動法による研究を実施し，その中間報告を発表した.[1-3] 1982年9月末までに，近距離被爆者の子供11,534人及び遠距離被爆者の子供9,092人を対象に最高30種類の蛋白質について，変異型を検索した．検査した蛋白質の数は子供により異なった．Harris ら[4] 及びNeel ら[1] と同じ手順により，遺伝子座相当テスト数を計算すると被爆群では543,664，対照群では386,706となった．被爆群では3例，対照群では2例の新しいミュータントを検出し，世代当たり遺伝子座当たりの突然変異率は各々$0.55 \times 10^{-5}$ 及び$0.52 \times 10^{-5}$ となったので，現在までのところ両親の原爆被爆による測定可能な遺伝的影響は認められないことは明らかである．更に，酵素活性の減少を指標として，突然変異を検出する研究を1979年に開始し，現在までのところ両群合計して49,076遺伝子座相当テストを行ったが，突然変異は検出されていない.[5]

今回の一連の報告では，原爆被爆者及びその子供の電気泳動法による研究の結果について記述し，日本人集団における蛋白質変異の頻度と種類を明らかにする上で寄与したい．本報告では，PGM2 に関する我々の研究の結果について述べる．

## 材料及び方法

**サンプル及び家族調査.** この一連の報告集の大部分において，対象者は，放影研で行われている二つの調査，すなわち，成人健康調査及び原爆被爆者の子供の蛋白構造に対する原爆放射線の遺伝学的影響の調査（$F_1$ 遺伝生化学調査）のメンバーである．成人健康調査は，広島・長崎の原爆被爆者と，性・年齢構成をこれらの被爆者とマッチさせて選んだ非被爆者をコントロールとして含む約20,000人（オリジナル数）についての医学的観察プログラムである．成人健康調査対象者は2年に1回の臨床検査に関しては，（原爆被爆歴を除き）無作為に選ばれているが，本調査では，ABCC-放影研遺伝学調査[6,7] の対象者である子供の親に優先順位を与えて抽出した．本研究のため電気泳動法による検査を受けた成人健康調査対象者4,649人を“成人”集団と呼ぶ．この集団は

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 nonexposed individuals were combined. Inasmuch as results of earlier electrophoretic studies performed on 4,029 samples from the Adult population have already been reported,[8-12] 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 variant is counted on the basis of the entire number of individuals examined, which differs from system to system.

広島3,066人，長崎1,583人の対象者で構成されるが，これらの対象者はすべて原爆投下以前に生まれているので，その蛋白質構造は放射線被曝による影響を受けていないと考えられる．そこで被爆者及び非被爆者から得られたデータは一つにまとめた．成人集団中の4,029人に関しては，以前に行われた電気泳動法による研究結果が既に報告されているので，[8-12] 本報及び続報においては，それら5報の発表後に検査した620人に認められたまれな変異型の特徴についてのみ述べる．しかし，各変異型の数は，蛋白質ごとに異なる総被検者数を基に計算する．

Members of the $F_1$-BGS population constitute a part of the $F_1$ Mortality Study comprising two groups of children: children of proximally exposed A-bomb survivors 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 identification (ID) number only.

$F_1$遺伝生化学調査のメンバーは，$F_1$死亡率調査の対象者の一部であり，次の2群の子供から成っている．すなわち広島・長崎の近距離原爆被爆者の子供（両親又は片親が爆心地から2,000 m未満で被爆したもの）と，そのコントロールとして選ばれ第1グループの子供と性・年齢別の構成を一致させた遠距離被爆者の子供（両親又は片親が爆心地から2,500 m以遠で被爆したもの）である．緒言で述べたとおり，親の被爆歴に関連した測定可能な遺伝的影響は認められなかったので，両群から得たデータはプールし，その集団を“子供”集団と呼ぶことにした．その集団のうち約30%は同胞であり，メンバーの親の中には成人集団に含まれるものもいる．被検者間の関係を知っていると起こるかもしれない偏りを防ぐため，すべての実験は，確認番号のみを付けたサンプルを用いて行った．

Since kinship is involved among some individuals in the Adult and 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

成人集団と子供集団の間，及び子供集団内部には，血縁関係をもつものがあるため，本報及び続報においては下記の手順で結果を報告する．すなわち，第一に各集団に検出されたまれな変異型については，得られた結果をそのまま報告する．次いで両集団から得たデータをプールし家族ごとにまとめ，それぞれの家族からただ1人を選び血縁関係のない個人で構成される“代表者”と呼ばれる集団を作る．対立遺伝子頻度はこの集団に基づいて計算する．成人集団4,029人から得たデータは既に発表されている

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 that 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 a variant was of a genetic nature. As the purposes 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,[8] while in the case of the Child population, the purpose was specifically to examine parents.[1] 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 population, but were excluded when frequencies were calculated since the family representative was the parents.

**Nomenclature of Rare Variants.** Rare variants were named as a rule, according to the method of Ferrell et al.[8] When variants detected among residents of Hiroshima or Nagasaki showed mobility similar to that of a specific variant reported in the literature, HR (cases first detected in Hiroshima) or NG (cases first detected in Nagasaki) was added to the conventional figures or characters of the variant reported in the literature. When variants with similar mobility could not be found in published reports, the variant was named according to the existing nomenclature of the isozyme of the protein and HR or NG was added. When many types of variants were detected, they were classified in

ので，代表者集団のメンバーを選ぶときには親を優先した．次に，成人集団に親のいない者の中から同胞のいない子供を選び，同胞のいる子供の場合には最初に検査を受けた者を選んだ．数人の同胞が同一日に検査を受けた場合は，第一番目に検査を受けた者を家族の代表として選んだ．

変異型が遺伝的なものかどうかを確認するために，家族調査を行った．先に述べた二つの調査の目的が異なったため，両集団における家族調査の方法も異なっていた．成人集団に関しては発端者と同一の変異型が検出されるまで家族調査を行ったが，[8] 子供集団の場合には両親を検査することが特に目的であった．[1] どちらの集団に関しても，協力的な家族もいたが，そうでない家族もおり，また，家族のメンバーが広島・長崎に居住しない場合もあった．親が成人集団に属し，子供が子供集団に属していたため家族調査が自動的に完了することもあった．しかし，子供集団の子供の両親として家族調査を受けた者が，たまたま成人健康調査の対象者であったとしても，その検査結果は成人集団の検査結果には算入しなかった．一方，成人集団の家族として検査を受けた者が，たまたま子供集団に属する子供であった場合には，そのデータは子供集団に関するデータとして採用したが，家族代表は両親なので頻度を計算する際には除外した．

**まれな変異型の命名．** まれな変異型の命名は，Ferrell ら[8] の方法に原則的に従うことにした．広島・長崎に居住する人に検出された変異型が，文献上既に報告されている特定の変異型に類似した移動度を示す場合には，その文献中の変異型に通常使用されている数字又は文字に HR（広島で最初に検出されたもの）又は NG（長崎で最初に検出されたもの）を付けた．それに類似した移動度をもつ変異型が発表された文献に見当たらない場合には，現行の蛋白質又はアイソザイムの命名法に従って変異型の命名を行い，HR 又は NG を付けた．変異型の種類が多いときは，検出

order of detection. Inasmuch as there are many examples of variants with identical mobility on starch gel electrophoresis subsequently shown to be nonidentical on the basis of polyacrylamide gel electrophoresis, isoelectric focusing (IEF), and/or enzyme kinetic determinations, even when the variant detected has the same electrophoretic mobility as that of a known variant on the same gel, it is unwise to assign such a variant the same name as that given to the variant discovered in an individual of a completely different population until the amino acid substitution of each variant is known. Therefore, this previously established nomenclature system will be used here.

された順番をもつけて区別した．澱粉ゲル電気泳動法では同一の移動度を示す変異型でも，ポリアクリルアミドゲル電気泳動法，等電点分離法若しくは酵素活性測定法では同一物ではないことを示す例が多いので，新しく検出した変異型が同一ゲル上で，既知の変異型と同じ移動度を示したとしても，それぞれの変異型のアミノ酸置換が明らかになるまでは，新しい変異型に全く異なる集団の一員に検出された変異型と同じ名称を付けるのは適当ではない．したがって，本研究では従来の命名法を使用した．

**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 populations, but most samples obtained from the latter was drawn into acid citrate dextrose (ACD) solution (Formula A of Beutler[13]). The Hiroshima blood samples were stored at 4°C for 1-3 days and separated into plasma and erythrocytes by centrifugation at 1,200×g for 20 minutes. After the buffy coat was removed, the erythrocyte layer was mixed with an equal volume of 0.85% NaCl solution, agitated for one minute and centrifuged at 1,200×g for five minutes. This was repeated two times, though the third centrifugation was continued for 20 minutes. The plasma and washed erythrocyte layer were, as a rule, apportioned into aliquots of 0.5-1.0 ml each in small polyethylene vials and cryopreserved in liquid nitrogen. When tests could be conducted immediately, fresh samples were used, and when tests could be conducted within two weeks, samples were preserved at −70°C. 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.

**標本の調製と保存．** 成人集団の大部分及び子供集団の一部から得た血液標本には，抗凝固剤としてアンモニウム-カリウム蓚酸塩を用いたが，子供集団から得た標本の大部分には，クエン酸グルコース（ACD）溶液を用いた（Beutler[13] のA処方）．広島の血液標本は1～3日間，4°Cで保存した後，20分間1,200×gで遠心分離し，血漿及び赤血球に分離した．バフィコートを除いた後，赤血球層を同量の0.85% NaCl溶液と混ぜ，1分間撹拌し1,200×gで5分間遠心分離した．これを2回繰り返したが，3回目の遠心分離は20分間行った．血漿と洗浄した赤血球層は，通常小さなポリエチレン・バイアルに各々0.5～1.0mlずつ分注し液体窒素中に冷凍保存した．直ちに検査を行うことができる場合には，新しい標本を用い，2週間以内に検査が可能な場合は標本を−70°Cで保存した．長崎で入手した標本の大部分は直ちに血漿と赤血球に分離した．血漿及び洗浄した赤血球層を−70°Cで凍結させ，ドライアイスで冷却しつつ広島へ輸送した．酵素活性測定法による調査を1979年に開始してからは，長崎で入手した全血液を4°Cで1～3日間保管し，氷詰めで広島へ送り，上述した広島の標本の場合と同様に調製した．標本の輸送及び保存の条件は，スクリーニングの期間中必ずしも同一ではなかったが，注意深く検討を行ったところでは，電気泳動上の結果には影響を及ぼしていなかった．再検査と変異型の相互比較に

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.

For hemolysate preparation, an equal volume of distilled water was added to the erythrocytes, and the mixture strongly agitated for 10 sec. After standing for 2 minutes, a volume of toluene equal to that of the original packed cells was added, agitated for 2 minutes and centrifuged at 1,200×g for 20 minutes. The toluene layer was sucked off, the hemolysate layer centrifuged at 30,000×g for 20 minutes to precipitate cell debris, and the supernatant hemolysate was collected.

**Vertical Starch Gel Electrophoresis.** Electrophoresis was conducted at 4°C and 7 V/cm for 20-23 hours employing Tris-EDTA-Maleic acid-$MgCl_2$ (TEMM) buffer, pH 7.4 of Spencer et al,[14] 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, USA) 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.[14] However, 6,928 samples from children subjected to tests since September 1978 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,[15] 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 one hour.

**PGM Activity Measurement.** Methods employed for determination of the PGM activity were the same as those described in a separate paper[16] concerning a low activity variant of PGM1 which in principle are based on the methods recommended by Beutler[13] and Beutler et al.[17] Mean ± standard deviation (SD) of PGM activity

は液体窒素中に保存した標本を先ず使用したが，可能な場合は新しく採血した血液を用いた．低活性変異型のバンドでも液体窒素中に7～8年間保存した標本を用いて明確に検出することができたし，30種の蛋白質についてそれらの標本の変異型バンドの移動度は，新しい標本のものと差異はなかった．

溶血液作成のためには赤血球に同量の蒸留水を加え，10秒間強く撹拌した．2分間放置した後，最初に遠心した赤血球と同量のトルエンを加え，2分間撹拌し1,200×gで20分間遠心分離した．トルエン層を吸引除去し，細胞の破片を沈澱させるため溶血液層を20分間30,000×gで遠心分離し，溶血液上清を収集した．

**垂直澱粉ゲル電気泳動．** Spencer ら[14] の Tris-EDTA-Maleic acid-$MgCl_2$ (TEMM) 緩衝液 (pH 7.4) をブリッジ緩衝液として，その1/15の希釈溶液 (pH 7.4) をゲル緩衝液として用い，電気泳動を4°C，7 V/cmで20～23時間行った．第1スクリーニングには，濃度13.3%の Electrostarch (Electrostarch 社，米国 Wisconsin 州 Madison 市) ゲルを主に使用したが，変異型の比較のためには Connaught-starch ゲル (13.3%) を使用した場合もある．Phosphoglucomutase (PGM) 活性による染色は，Spencer ら[14] の方法により実施した．しかし，子供の標本のうち1978年9月以降検査をした6,928標本では，寒天オーバーレイの代わりにブラシでゲルの表面に染色溶液を塗ることにより直接に染色を行った．Phosphopentomutase (PPM) 活性に基づく染色法は，リボース5燐酸塩を基質として使用する Quick ら[15] の不安定燐酸塩検出法により行った．PPM 反応は寒天オーバーレイの代わりにゲルを反応溶液に浸し，37°C で1時間インキュベートして行った．

**PGM 活性の測定．** PGM 活性の測定のため使用した方法は，PGM1 の低活性変異型に関する別報[16] に述べたものと同じであるが，その方法は原則的には Beutler[13] 及び Beutler ら[17] が推薦した方法に基づく．広島の標本 (n＝191) 及び長崎の標本 (n＝195) の

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.

## RESULTS

In the previous paper,[10] the data obtained by electrophoresis using both a TEMM buffer and a histidine-citrate discontinuous buffer system by Fildes and Harris[18] were scored, 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 examinations 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 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 Adult population, were encountered in the Child population. Figure 1 shows the starch gel electrophoretic patterns of these five variants and Figure 2 is a diagram showing them. All showed PPM activity, confirming them as allozymes of PGM2 (Figure 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.

PGM 活性の平均値±標準偏差(SD)は各々1.82±0.23 IU/gHb(ヘモグロビンのg当たりの国際標準単位)及び 1.85±0.19 IU/gHb であり，それらの PGM1 と PGM2 の表現型はともに1であった．まれな変異型を示した発端者の PGM 活性は可能であればいずれの場合も測定し，その家族についても電気泳動及び PGM 活性の測定を行った．各変異型の特性を述べるときにそれらの活性についても言及する．

## 結　果

前回の報告[10]では，TEMM 緩衝液を用いた電気泳動により得たデータも，Fildes 及び Harris[18] のヒスチジン-クエン酸塩不連続緩衝液系を使用した電気泳動によって得たデータもともにデータとして用いた．しかし，ヒスチジン-クエン酸塩不連続緩衝液系では，移動度の遅い PGM2 変異型を見逃すおそれのあることが分かったので，本報では TEMM 緩衝液による検査結果のみ述べる．したがって，成人集団及び子供集団において，PGM2 の検査数は，この研究全体の対象者数より少ない．

成人集団中2,534人及び子供集団中14,592人の検査では，多型の頻度で存在するありふれた変異型は観察されなかった．

まれな変異型1種類が成人集団に検出され，5種類の変異型が子供集団に検出された．そのうちの一つは成人集団のものと同じであった．図1はこれら5種類の変異型の澱粉ゲル電気泳動パターンで，図2はそれらを模式図で示したものである．これらはすべて PPM 活性を示し，それらが PGM2 のアロザイムであることを明らかにした(図3)．

表1には各集団において様々な表現型をもつ人の数を示す．表現型を明確に読み取ることができなかったものは“no type”とした．代表者集団を選ぶときには“no type”は除外した．これらの者を除き，代表者をサンプル及び家族調査の項で述べた方法により選んだ．したがって，家族の中に正常型をもつ者と変異型をもつ者がいる場合，前者を代表者集団に選び，後者を除外した場合もある．家族調査の結果を表2に示す．

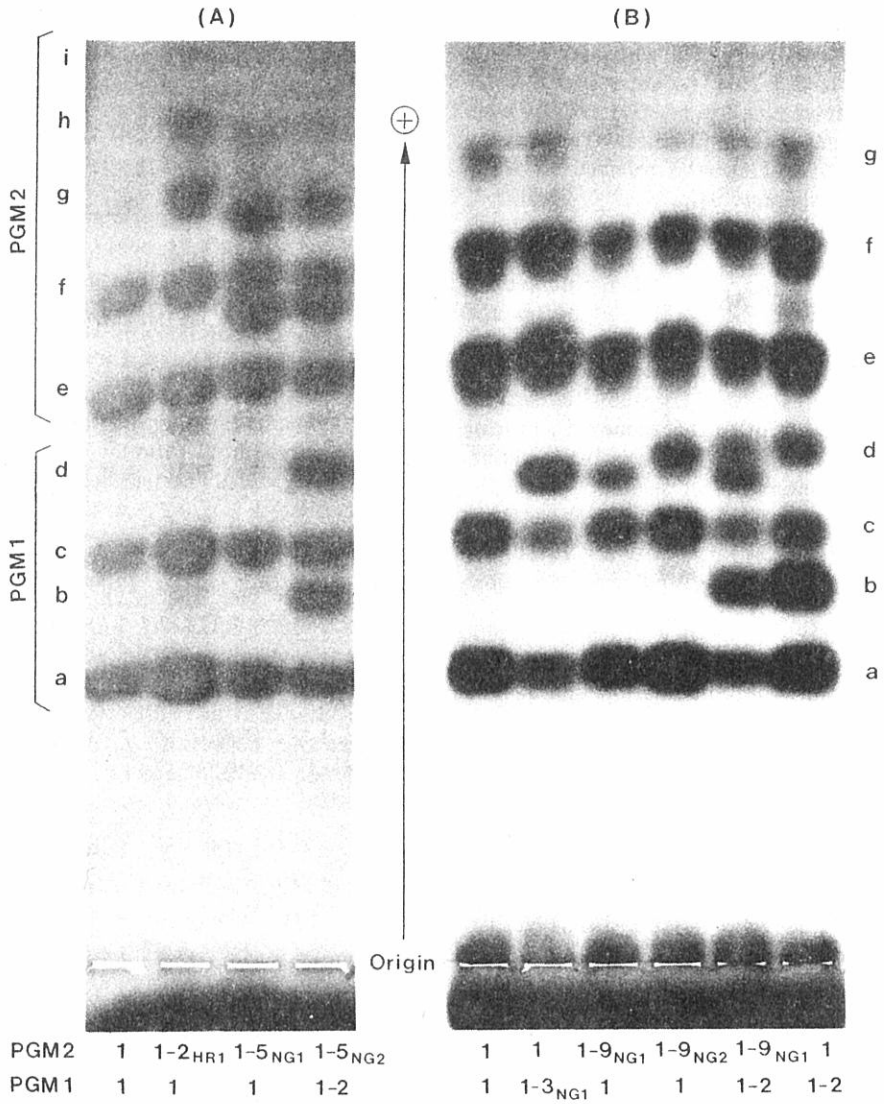


*Figure 1. Five types of PGM2 variants migrating faster (A) and slower (B) than PGM2 1 on electrostarch gel, stained for PGM activity.*

図1　Electrostarch で作ったゲル上で PGM2 1 より速く（A），遅く（B）移動する PGM2 の変異型5種を PGM 活性で染色したもの．

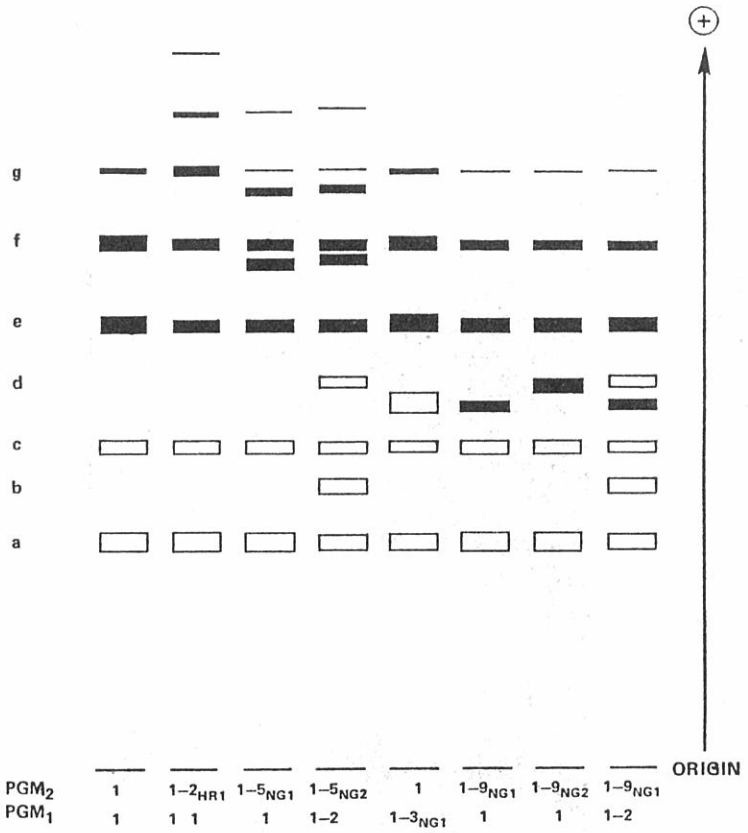


*Figure 2. Diagram of PGM2 variants on starch gel employing TEMM buffer, pH 7.4 (Gel buffer is 1/15 dilution of bridge buffer).*

図2　TEMM 緩衝液，pH 7.4を用いた澱粉ゲル上での PGM2 の変異型の模式図（ゲル緩衝液はブリッジ緩衝液を1/15に希釈したもの）.

**PGM2 $2_{HR1}$**

In a female Hiroshima child with ID No. [redacted], five bands of PGM2 were 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 $f=e \geqslant g > h > i$. Mobility of these bands was very similar to that of the five allozyme bands of PGM2 1-2 detected by Hopkinson and Harris[19] 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.

**PMG2 $2_{HR1}$**

広島の子供集団のメンバーで ID 番号[redacted]の女性には，PGM2 のバンドが5本検出された．すなわち，3本の *e*-，*f*-，*g*- バンドの陽極側に *h*- バンド及び *i*- バンドを認めた．PGM 及び PPM 活性で染色したこれらのバンドの染色度は $f=e \geqslant g > h > i$ であった．これらのバンドの移動度は，Hopkinson 及び Harris[19] が英国及びアフリカに住む黒人から検出した PGM2 1-2 の5本のアロザイムのバンドの移動度と非常によく似ていた．我々の命名法に従いこの変異型（表2の No. 1）を PGM2 $2_{HR1}$ と命名し，家族調査により遺伝的変異型であることを確認した．発端者の母親，叔父（母親の弟）及び弟には，発端者と同一の変異型を認めたが，もう1人の男の同胞，もう1人の叔父（母親の弟）及び伯母（母親の姉）は PGM2 1 であった．

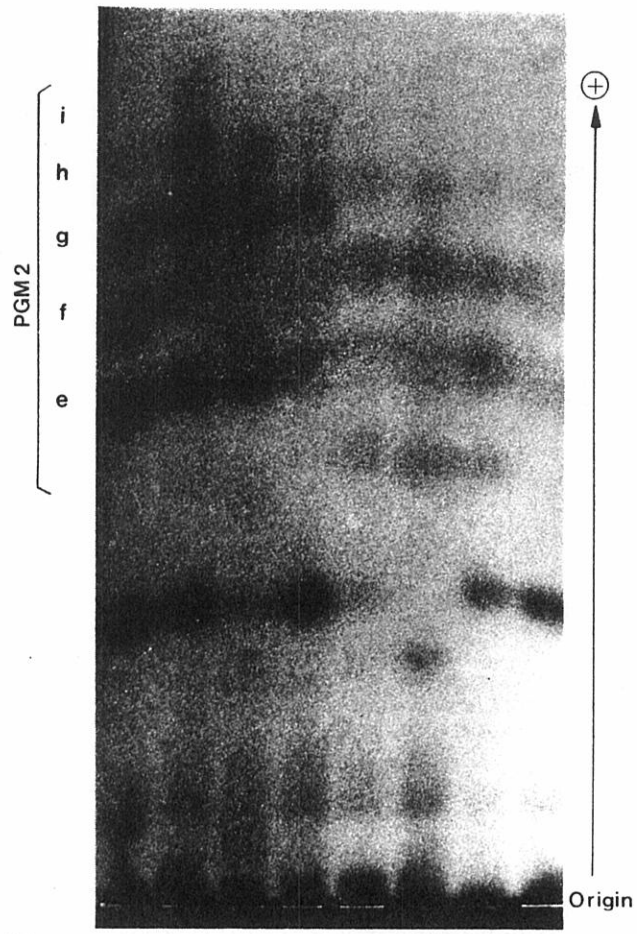


*Figure 3. Five types of PGM2 variants stained for phosphopentomutase activity, encountered in the Hiroshima and Nagasaki populations.*

図3　広島・長崎の集団に検出された PGM2 の変異型5種をホスホペントムターゼ活性で染色したもの.

TABLE 1 VARIOUS PHENOTYPES OF PGM2 ENCOUNTERED AMONG JAPANESE OF TWO POPULATIONS AND THE REPRESENTATIVE POPULATION*
表1 日本人2集団及び代表者集団*で検出したPGM2の様々な表現型

| Phenotype | Population | | | | |
|---|---|---|---|---|---|
| | Adult | Child | Representative | | |
| | | | Combined | Hiroshima | Nagasaki |
| 1 | 2,529 | 14,577 | 11,825 | 6,673 | 5,152 |
| $1\text{-}2_{HR1}$ | 0 | 1 | 1 | 1 | 0 |
| $1\text{-}5_{NG1}$ | 0 | 1 | 0 | 0 | 0 |
| $1\text{-}5_{NG2}$ | 0 | 1 | 1 | 0 | 1 |
| $1\text{-}9_{NG1}$ | 3 | 7 | 6 | 4 | 2 |
| $1\text{-}9_{NG2}$ | 0 | 1 | 0 | 0 | 0 |
| No type** | 2 | 4 | 0 | 0 | 0 |
| Total | 2,534 | 14,592 | 11,833 | 6,678 | 5,155 |

*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.
代表者集団は，成人集団及び子供集団から選択した血縁関係のない対象者により構成される．成人集団及び子供集団で検出された変異型が，代表者集団で必ず検出されるとは限らない．この集団の定義に関しては本文参照．

**The phenotype could not be read clearly.
表現型が不明のもの

TABLE 2 SUMMARY OF THE RESULTS OF FAMILY STUDIES OF VARIANTS OF PGM2 ENCOUNTERED IN THE CHILD POPULATION
表2 子供集団で検出したPGM2の変異型の家族調査結果の要約

| Propositus | | | | | Mother | Father | Other family member | Comments |
|---|---|---|---|---|---|---|---|---|
| Variant No. | City | Variant type | ID No. | Sex | | | | |
| 1 | H | $2_{HR1}$ | [redacted] | F | ♀ | ♂ | | *1 |
| 2 | N | $5_{NG1}$ | | F | ♀ | ♂ | ♂Brother | pI=5.9 |
| 3 | N | $5_{NG2}$ | | F | ♀ | ♂ | | pI=5.8 |
| 4 | N | $9_{NG1}$ | | F | ♀ | Dead | ♂Brother | |
| 5 | N | $9_{NG1}$ | | M | | | | |
| 6 | H | $9_{NG1}$ | | M | ♀ | ♂ | | |
| 7 | H | $9_{NG1}$ | | M | ♀ | ♂ | ♀Sister | |
| 8 | H | $9_{NG1}$ | | M | ♀ | ♂ | ♂Brother | |
| 9 | N | $9_{NG1}$ | | M | ♀ | ♂ | | |
| 10 | H | $9_{NG1}$ | | M | ♀ | ♂ | ♂Brother | |
| 11 | N | $9_{NG2}$ | | M | ♀ | ♂ | ♀Sister | Mutation |

♀,♂ Heterozygote for variant alleles at *PGM2* locus
*PGM 2* 遺伝子座における変異型対立遺伝子のヘテロ接合体

♀,♂ Homozygote for normal *PGM2*1*
正常 *PGM 2**1 のホモ接合体

][ Siblings 同胞

*1 For results of examination on other family members, see text.
他の家族構成員の検査結果に関しては本文参照

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

Two fast variants (No. 2 and No. 3 in Table 2) whose mobilities were almost 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.[20] Variant bands moved so close to *f*- and *g*-bands that they were almost in contact with the cathodal side of *f*- and *g*-bands and the third band moved to the anodal side of the *g*-band. The intensities of these three bands were similar respectively to those of heterozygous *e*-, *f*-, and *g*-bands of these same individuals. Electrophoresis of the hemolysates from these two 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 (IEF), 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 $5_{NG2}$.[21] In family studies, the mother of the propositus showing PGM2 1-$5_{NG1}$ showed the same variant, and the father and a brother showed PGM2 1; the 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*, and *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) Adult population. The band moved to the equal position of the major band of PGM1 $3_{NG1}$ and slightly more cathodally than *d*-band of PGM1 on the Electrostarch gel.[10] However, 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 $3_{NG1}$,[10]

### PGM2 $5_{NG1}$ 及び PGM2 $5_{NG2}$

移動度の速い変異型で，澱粉ゲル上の移動度がほぼ等しいものが2種類（表2のNo.2及びNo.3）長崎の子供集団の女性2人に検出されたが，この2人には血縁関係はなかった．PGM活性に基づいて染色した変異型バンドの移動度及び染色度はParringtonら[20]が発表した模式図中のPGM2 1-5と類似していた．変異型バンドは*f*-バンド及び*g*-バンドに非常に接近して移動し，*f*-バンド及び*g*-バンドの陰極側にほとんど接触するほどであり，第三番目のバンドは*g*-バンドの陽極側に移動した．これら3種類のバンドの染色度は各々，同一対象者のヘテロ接合型になっている*e*-バンド，*f*-バンド及び*g*-バンドに類似していた．TEMM緩衝液を使用し，同一の澱粉ゲル上で，これら2人の子供から得た溶血液の電気泳動を数回繰り返したが，バンドの分離が非常に良かったとき初めてこれらの変異型の移動度に微妙な差異が認められた．一方，ポリアクリルアミド薄層ゲル等電点分離法（IEF）では，澱粉ゲルでは移動の遅かった変異型No.2の主バンドの等電点（pI）が5.9であり，変異型No.3の場合は5.8であった．この実験において*e*-バンドのpIは6.0であった．これらの変異型のうち前者をPGM2 $5_{NG1}$，後者をPGM2 $5_{NG2}$とした．[21] 家族調査においてはPGM2 1-$5_{NG1}$を示した発端者の母親は同一の変異型を示し，父親と兄弟はPGM2 1を示した．PGM2 1-$5_{NG2}$を示した発端者の父親は娘と同一の変異型をもっており，母親はPGM2 1を示した．

### PGM2 $9_{NG1}$

型判定が問題なく行われた2,532人（広島　1,299人，長崎　1,233人）の成人集団のうち，広島では2名，長崎では1名にPGM2の3本のアロザイムバンド*e*, *f*, *g*のほかに移動度が遅く，染色度の弱い1本のバンドが検出された．そのバンドは Electrostarch ゲル上ではPGM1 $3_{NG1}$の主バンドと同じ位置で，PGM1の*d*-バンドよりもわずかに陰極側の所に移動した．[10] しかし，Connaught-starch ゲル上では，澱粉のlot番号によって，PGM1 $3_{NG1}$の主バンドよりわずかに陰極側の所に移動することがあった．Electrostarch ゲルを使っても，Connaught-starch ゲルを使っても副バンドは観察されなかった．これらの変異型のうち1例は，以前報告した成人集団に検出されたものであり，バンドの染色度は弱かったけれど移動度はPGM1 $3_{NG1}$と類似していたため，PGM1 $3_{NG2}$と命名していたものである．[10] その変異型は

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 and PPM activities 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.[22] 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 and PPM activities. 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. 4-10) 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 & 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 the *d*-band between the *c*-band and *d*-band under excellent electrophoretic conditions (Figures 1 & 2), but when good separation could not be obtained due to a slight difference in 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 two children were siblings whose mother had PGM2 1 and father was deceased.

PGM1 ではなく PGM2 の変異型であるかもしれないという Hopkinson 博士の示唆に従い，PPM 活性を測定したところ陽性の結果を得て，その推測が正しいことを確認した．更にこの対象者の *e*- バンド，*f*- バンド及び *g*- バンドの染色度は PGM 活性の場合にも PPM 活性の場合にも正常型のものより弱いことが認められたので，ヘテロ接合型であることが示唆される．この変異型の移動度は，Blake 及び Omoto[22] が Papua New Guinea で検出した PGM2 9 と類似していたので，PGM2 $9_{NG1}$ と改名した．したがって，上記 3 例の表現型は PGM2 1-$9_{NG1}$ である．PGM 活性及び PPM 活性で染色すると PGM2 $9_{NG1}$ のバンドの染色度は *e*- バンド及び *f*- バンドより弱く，*g*- バンドより強かった．PGM 活性の場合，$9_{NG1}$ のバンドの染色度は，ホモ接合の *c*- バンドより著しく弱かった．

型判定のできた子供集団の14,588人（広島7,594人，長崎6,994人）のうち，広島の子供 4 人，長崎の子供 3 人にも PGM2 1-$9_{NG1}$ を検出した（変異型 No. 4 ～ 10）．長崎の子供 3 人のうち 2 人は同胞であった（変異型 No. 4 及び 5）．PGM2 1-$9_{NG1}$ が検出された両集団の対象者10例のうち，8 例では PGM1 の表現型は 1 であり，2 例では 1-2 であった．後者の例，すなわち PGM1 1-2 及び PGM2 1-$9_{NG1}$ を示すサンプルでは，電気泳動条件が優れていたときは，PGM2 $9_{NG1}$ のバンドは *c*- バンドと *d*- バンドの間で，*d*- バンドに近いところに離れて見られるが（図 1 及び 2），澱粉の lot 番号又は電気泳動条件のわずかな違いのため明確に分離しないときには，*d*- バンドが正常のものより広く，その染色度が *b*- バンドと同様か又は *b*- バンドを上回ることを除いては，PGM1 1-2 及び PGM2 1 と比べ差異はほとんど認められなかった．代表者集団における *PGM2*9NG1* 対立遺伝子の頻度は0.00025であった．

成人集団に属する発端者のうち長崎の 1 例については家族調査を実施することができなかった．成人集団に属する他の広島の 2 例の場合は，各々の息子 1 人が母親と同一の変異型をもっていた．子供集団の 7 人のうち，5 人の発端者に関しては両親を検査し，どちらかの親に PGM2 $9_{NG1}$ を検出し（表 2），変異型の遺伝的伝達を確認した．他の 2 人の子供は同胞で，母親は PGM2 1 をもっており，父親は死亡していた．

Although the intensity of allozyme band of PGM2 $9_{NG1}$ was weak, PGM activity in 8 among 10 subjects in whom PGM 2 $9_{NG1}$ 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 IEF, 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 pI values of PGM2 $9_{NG1}$, PGM2 $9_{NG2}$, PGM2 1, PGM1 $3^{-}_{NG1}$, and PGM $3^{+}_{NG1}$, being 6.5, 6.2, 6.0, 5.9, and 5.8, respectively.[21,23] 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 band 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 and 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, and C typing

PGM2 $9_{NG1}$のアロザイムバンドの染色度は弱かったが，PGM2 $9_{NG1}$が検出された10例のうち8例の PGM 活性は，正常型（PGM1 1 及び PGM2 1）の92％に当たる1.69IU/gHb（SD＝0.21IU/gHb）という平均値を示しており，これは正常値としての範囲内にあるものと考えられる．

**PGM2 $9_{NG2}$**

PGM2 $9_{NG1}$に類似した移動度をもつ変異型バンドが，長崎の近距離被爆者を両親にもつ1960年生まれの男子に検出された．PGM2 $9_{NG2}$と命名されたこの変異型バンドは*d*-バンドと同位置，又はわずかにその陰極側に移動したが，PGM2 $9_{NG1}$のバンドよりわずかに陽極側に移動したので両者は区別された．PGM2 $9_{NG1}$, PGM2 $9_{NG2}$, PGM1 $3_{NG1}$及び*d*- アイソザイムのバンドの移動度はゲル作成のために用いた澱粉の種類及び lot 番号により異なった．それらの陽極側への移動度は，$d \geqslant$PGM2 $9_{NG2}$ >PGM1 $3_{NG1} \geqslant$PGM2 $9_{NG1}$ であった．しかし，薄層ポリアクリルアミドゲル等電点分離法では，PGM2 $9_{NG1}$及びPGM2 $9_{NG2}$はPGM2 1 の主バンドの陰極側にバンドを形成し，PGM1 $3_{NG1}$は陽極側にバンド形成した．PGM2 $9_{NG1}$, PGM2 $9_{NG2}$, PGM2 1, PGM1 $3^{-}_{NG1}$及び PGM1 $3^{+}_{NG1}$の等電点は各々約6.5, 6.2, 6,0, 5.9, 5.8 であった．[21,23] したがって各アイソザイムは明確に区別される．この変異型のバンドの PGM 活性染色度はホモ接合の *c*- バンドのものよりわずかに弱く，PGM2 $9_{NG1}$のバンドのものよりは明らかに強かった．発端者の *e*- バンド，*f*- バンド及び *g*- バンドの PGM 活性並びに PPM 活性は正常の PGM2 1 に比べて弱かった．したがって発端者の表現型は PGM1 1 及び PGM2 1-$9_{NG2}$であった．PGM2 1-$9_{NG2}$ において変異型バンドの染色度は *e*- バンドよりわずかに弱く，*f*- バンドより強かった．発端者の PGM 活性は 1.79IU/gHb で正常であった．

その発端者の直接の家族には変異型バンドは検出されず，両親，弟，妹はすべて PGM1 1 及び PGM2 1 を示した．家族の血液型及び蛋白質型を表3に示す．6か月間に2回新たに入手した血液標本を用い，発端者及び両親の血液型及び PGM2 を含めた蛋白質型を繰り返し調べたところ，同じ結果を得た．第二回目に入手した標本を用いて HLA-A, B, C の

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 gamma rays and 2 rad for neutrons, while that of the father was 10 rad for gamma rays.[24] According to the calculation method of Kerr et al,[25] gonadal dose to the mother was 91.6 rad gamma rays and 0.24 rad neutrons and that of the father was 6.5 rad gamma rays. Hematological data of the propositus are normal and he has been healthy.

型決定を行った．法律上及び生物学上の親子関係の資料に不一致はなかった．同時に行われた通常の染色体検査法及びC-バンド法，Q-バンド法を用いた染色体検査においても，真の生物学的親子関係以外のものを示唆する矛盾した証拠は認められなかった．したがって，発端者に検出された変異型は，いずれか一方の親に発生した突然変異に帰因する新しいミュータントであると考えられる．母親の受けた空気線量はガンマ線が226rad，中性子線が 2 rad であり，父親の場合はガンマ線が 10rad であった．[24] Kerr ら[25] の計算法によると，母親の生殖腺線量はガンマ線 91.6rad，中性子線 0.24rad であり，父親の場合はガンマ線 6.5rad である．発端者は，血液学的データも正常で，健康であった．

TABLE 3 BLOOD, PROTEIN, AND HLA TYPES OF THE PARENTS AND THE PROPOSITUS HAVING A PUTATIVE MUTANT OF PGM2 (PGM2 $9_{NG2}$)

表 3 PGM2 のミュータントと考えられるもの（PGM2 $9_{NG2}$）をもつ発端者と両親の血液型，蛋白質型及び HLA 型

| | 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 ($\alpha_1$-AT) | 1-2 | 1-1 | 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 | $1\text{-}9_{NG2}$ | 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 ($\alpha_1$-antitrypsin), HP = haptoglobin, ACP1 = acid phosphatase-1, ADA = adenosine deaminase, ESD = esterase D, GOT1 = cytoplasmic glutamate-oxaloacetate transaminase, GPT = glutamate-pyruvate transaminase, 6PGD = 6-phosphogluconate dehydrogenase, PGM1, PGM2, PGM3 = phosphoglucomutase-1, -2, -3, HLA = human leukocyte antigen.

略語：PI＝プロテアーゼ抑制因子（$\alpha_1$-抗トリプシン），HP＝ハプトグロビン，ACP1＝酸性ホスファターゼ-1，ADA＝アデノシンデアミナーゼ，ESD＝エステラーゼD，GOT1＝細胞質グルタミン酸-オキザロ酢酸トランスアミナーゼ，GPT＝グルタミン酸-ピルビン酸トランスアミナーゼ，6PGD＝6-ホスホグルコン酸デヒドロゲナーゼ，PGM1，PGM2，PGM3＝ホスホグルコムターゼ-1，-2，-3，HLA＝ヒト白血球抗原

## DISCUSSION

In the face of the well-known diversity of PGM1,[10,14,19,22,26-29] PGM2 is monomorphic in Caucasoid and most of Mongoloid populations,[30] though it is polymorphic in Negroid[19] and certain populations such as the Trio Indians,[31] aboriginals from central Australia,[32] and inhabitants of New Guinea.[22] The reported number of rare PGM2 variants is small and frequencies are low in the three human races. The difference in the two enzymes is especially large in Japanese. In a previous paper, Satoh et al[10] 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 with no variants being observed, despite their possible common origin through gene duplication.[30] The same phenomenon is again observed in this study in which the population is approximately six 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.[16] In Ishimoto's review,[33] a combined sample of 10,851 Japanese excluding our previously reported 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,[34] which are named PGM2 $5_{NG1}$ and PGM2 $9_{NG1}$ in this paper, no other PGM2 variants in Japanese have been described until recently Nishigaki[35] detected a variant similar to phenotype PGM2 1-5 of Parrington et al,[20] 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,[4] 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.[12] A higher mutation rate in Japanese was suggested as a possible explanation for the difference between the two populations with respect to PGM1 variation.

Recently, we reported that the conventional allele *PGM1*7* which is present in polymorphic

## 考　察

よく知られるとおり，PGM1 には変異が非常に多いが，[10,14,19,22,26-29] PGM2 は白人集団及び大部分のモウコ人集団[30]では多型性ではない．ただし黒人集団[19]及びTrioインディアン，[31]中央オーストラリアの原住民，[32]ニューギニアの住民[22]などの特定の集団においては多型性である．ヒトの3大人種において，PGM2 のまれな変異型の報告例数は少なく頻度は低い．この2種類の酵素の差は日本人において特に大きい．以前の報告で佐藤ら[10]は，日本人においてPGM1 は，変異型の種類も頻度も多様な酵素であるにもかかわらず，起源はこれと同じであり，遺伝子重複により作られた可能性もあり，[30]多型性ではなく変異型も認められていない PGM2 とは対照的であることを述べた．前回の約6倍の大きさの集団を対象とした今回の調査においても，同じ傾向が再度認められた．PGM2 のまれな変異型5種は，血縁関係のない11,833人を含む計17,126人のうち，14人に検出されたが，PGM1 のまれな変異型13種は103人に検出された．[16] Ishimoto[33] のまとめによると，我々が以前に報告した集団を除いて PGM1 と PGM2 について検査された日本人の合計10,851人中に，PGM2 のまれな変異型は報告されていなかった．本報告で PGM2 $5_{NG1}$ 及び PGM2 $9_{NG1}$ と命名された2種の PGM2 の変異型で著者らが前回の中間報告[34]で述べた変異型のほかには，日本人の PGM2 の変異型は最近まで報告されていなかった．直接的な比較研究は実施されていないが，Parrington ら[20]の表現型 PGM2 1-5，あるいは，恐らく，本対象集団の PGM2 1-$5_{NG1}$ 又は PGM2 1-$5_{NG2}$ に類似した変異型を最近になって Nishigaki[35] が検出している．

日本人集団と英国人集団[4]について，これら2種の酵素における変異を比較すると，集団構成に影響を及ぼす気候，大陸との地理的位置関係，集団の歴史，産業化の程度に関してこれら二つの島国は類似しているにもかかわらず，PGM1 の変異の程度は，日本人集団の方が非常に高かった．[12] PGM1 の変異に関する両集団の差異は日本人の突然変異率の方が高いことに帰因するかもしれないと示唆された．

最近我々は，日本人の間で多型の頻度で存在する[10]従来の対立遺伝子 *PGM1*7* を，*PGM1*7+* 及び

proportions in the Japanese[10] can be subtyped into *PGM1*7+* and *PGM1*7–*.[36] *PGM1*3NG1*, which is the most frequent rare variant *PGM1* allele in Japanese of Hiroshima and Nagasaki,[16] can also be subtyped into *PGM1*3NG1+* and *PGM1*3NG1–* by IEF, and family studies confirmed these pI subtypes as real alleles. Considering the distribution of the conventional alleles of *PGM1*7* and *PGM1*3* in the Pacific area, and the pI values of all of eight pI alleles, four of which are common to populations of all three races and four new alleles found in Japanese, we proposed an evolutionary phylogeny for the pI alleles of PGM1.[23] It is an extension of the hypothesis originally proposed by Carter et al[37] that "mutation and intragenic crossing-over developed a series of pI 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 is much higher than that for true mutation and increases with the number of alleles, 'apparent' mutation which is a combination of 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 pI variants of PGM2,[23] nor have other authors described such variants of PGM2.[38,39] Moreover no heterogeneity in sensitivity to heat denaturation of PGM2 isozymes was described by Scozzari.[29] 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.

*PGM1*7*－に分類することができると報告した.[36] 広島・長崎の日本人集団の示すまれな変異型 *PGM1* 対立遺伝子の中で最も頻度が高い[16] *PGM1*3NG1* も，IEF により *PGM1*3NG1*＋及び *PGM1*3NG1*－に分類することができ，更に家族調査によりこれらの等電点による亜型は真の対立遺伝子であることが確認された．太平洋地域における従来の分類による対立遺伝子 *PGM1*7* 及び *PGM1*3* の分布，及び三大人種すべてに共通な4種と日本人に新たに発見された4種を含む8種の等電点によって分類された対立遺伝子の pI 値を考慮し，我々は PGM1 の等電点対立遺伝子の進化の系統樹を提出した.[23] これは以前 Carter ら[37] が提案した「突然変異と遺伝子内乗り換えによって一連の等電点対立遺伝子が発生した」という仮説を拡張するものである．遺伝子突然変異とそれに続いて生じる遺伝子内乗り換えというこの仮説は，日本人における PGM1 の多様性を非常によく説明しているように思われる．乗り換えの起こる可能性は，真の突然変異の生じる可能性よりもずっと高く，また対立遺伝子の数が多くなるほど，その可能性は増大するので，真の突然変異と乗り換えの組み合わせである"見掛上の"突然変異は，対立遺伝子数の多い集団においては，非常に速く増加する．日本人においては，対立遺伝子 *PGM1*7* が多型の頻度で存在することが PGM1 の多様性に寄与しているように考えられる．

我々は1,000人を IEF で検査したが，その際に PGM2 の等電点の異なる変異型を検出できなかったし,[23] 他の研究者らもそのような変異型については報告していない.[38,39] 更に，Scozzari[29] も PGM2 アイソザイムの熱変性に対する感受性に不均一性があるとは述べていない．著者らの仮説によれば，日本人集団又は白人集団においては，遺伝子内乗り換えを起こすような PGM2 の第二の対立遺伝子が多型の頻度では存在しないために，PGM2 の見掛上の突然変異率は低くなると考えられる．

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