Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10725–10729, November 1993
Genetics

Intragenic recombination at the human phosphoglucomutase 1 locus: Predictions fulfilled

(phosphoglucomutase 1 mutations/phosphoglucomutase 1 phylogeny/molecular evolution/intragenic recombination)

NORIO TAKAHASHI[†‡] AND JAMES V. NEEL[§]

[†]Department of Genetics, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-Ku, Hiroshima 732, Japan; and [§]Department of Human Genetics, M4708 Medical Science II, University of Michigan, Ann Arbor, MI 48109-0618

Contributed by James V. Neel, June 17, 1993

ABSTRACT **In 1982, we advanced a phylogeny that attributed eight alleles of the phosphoglucomutase 1 locus (*PGM1*) to three independent mutations in a primal allele, followed by four intragenic recombination events involving these mutants [Takahashi, N., Neel, J. V., Satoh, C., Nishizaki, J. & Masunari, N. (1982) *Proc. Natl. Acad. Sci. USA* 79, 6636–6640]. The recent description of a cDNA probe for this locus [Whitehouse, D. B., Putt, W., Lovegrove, J. U., Morrison, K., Hollyoake, M., Fox, M. F., Hopkinson, D. A. & Edwards, Y. H. (1992) *Proc. Natl. Acad. Sci. USA* 89, 411–415] now renders it possible to test the validity of this phylogeny. cDNAs of *PGM1* reverse-transcribed from mRNAs obtained from Japanese individuals possessing eight different electrophoretically defined alleles (*PGM1*1+*, *PGM1*1−*, *PGM1*2+*, *PGM1*2−*, *PGM1*3+*, *PGM1*3−*, *PGM1*7+*, *PGM1*7−*) were amplified by PCR and the sequences were determined. Only three different base substitutions were identified when *PGM1*1+* was taken as the reference allele, as follows: an A to T transversion at residue 265, a C to T transition at residue 723, and a T to C transition at residue 1320. The second of these substitutions creates a *Bgl* II restriction enzyme site and the third creates a *Nla* III site. At the amino acid level, these substitutions alter amino acid 67 from Lys to Met, amino acid 220 from Arg to Cys, and amino acid 419 from Tyr to His, respectively. These mutations resulted in the electrophoretic properties defining *PGM1*7+*, the *PGM1*2+*, and the *PGM1*1−* alleles, respectively. Subsequent intragenic recombinational events resulted in the remaining four alleles. For two of these latter alleles (*PGM1*7−* and *PGM1*3−*), more than one type of intragenic crossover can produce the allele. These findings verify the predicted phylogeny and provide a case study in the evolution of complexity at a genetic locus.**

Phosphoglucomutase (PGM; α-D-glucose 1,6-phosphomutase, EC 5.4.2.2) catalyzes the interconversion of glucose 1-phosphate and glucose 6-phosphate. The results obtained from family studies with starch gel electrophoresis (SGE) to define the PGM of various kinds of human tissues have demonstrated that the PGM isozymes that are observed are encoded by three independent autosomal gene loci designated *PGM1*, *PGM2*, and *PGM3* (1). A polymorphism of human *PGM1* based on the presence of two alleles, *PGM1*1* and *PGM1*2*, has been observed in all the populations of the world thus far examined by SGE (1). Two additional alleles, *PGM1*7* and *PGM1*3*, have been encountered sporadically all over the world but, on the basis of current information, show local (but different) maxima in Asian–Pacific populations (2). In Japanese, the *PGM1*7* allele occurs with a frequency of 0.0140 and the *PGM1*3* allele occurs with a frequency of 0.0015 (3).

The phenotypes associated with the two common alleles *PGM1*1* and *PGM1*2* can each be subdivided by polyacrylamide gel isoelectric focusing (PAGIF) into two phenotypes; one, designated +, migrates more anodally than the other, designated − (4, 5). Thus, four alleles, termed *PGM1*1+*, *PGM1*1−*, *PGM1*2+*, and *PGM1*2−*, define the 10 common phenotypes. From the nonrandom association of the + and − attributes with the conventional alleles—namely, 1 vs. 2 and + vs. −, Carter *et al.* (6) postulated an allele phylogeny in which the four alleles could have arisen by a combination of two mutations and one subsequent intragenic recombination. We (7) have extended this isoelectric typing to the products of the two rarer alleles, *PGM1*3* and *PGM1*7*, as these were encountered in Japanese, and have demonstrated that these alleles can also be subclassified as + or −. Here also these properties are not associated at random with reference to the electromorphs under study. The additional four alleles were termed *PGM1*3+*, *PGM1*3−*, *PGM1*7+*, and *PGM1*7−*. On the basis of these facts plus the additive nature of the pI differences between the allele products and the geographical distribution of the alleles, an extended phylogeny could be constructed based on three mutations in a primal allele followed by four intragenic recombination events.

Recently, Whitehouse *et al.* (8) isolated the cDNA encoding the mRNA for a human *PGM1* allele and determined its full sequence. The cloning of this allele provided an opportunity for an empiric test of the validity of the proposed phylogeny. In the present communication, we report the sequences of DNAs amplified from the mRNA obtained from individuals whose subtypes were determined previously by electrophoresis. The sequence variations observed on the eight types of allelic products strongly support our proposed phylogeny.

SAMPLES

The blood samples on which this study was performed were obtained from individuals who had participated in a study of the occurrence of protein variants in the children of individuals exposed to the potential genetic effects of atomic bombs (9). Because no putative mutations involving the *PGM1* locus have been encountered in this study, the samples have been analyzed without reference to the radiation histories of either the individuals themselves or the parents of the individuals in question. In the previous studies, the *PGM1* types of the population were determined by SGE (3) and PAGIF (7). For this study, blood samples of known *PGM1* type were drawn, and peripheral B-lymphocyte cell lines were established by transformation with the Epstein–Barr virus. Subsequently, RNAs were extracted from the cultured cells by a modifica-

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Abbreviations: SGE, starch gel electrophoresis; PGM, phosphoglucomutase; PAGIF, polyacrylamide gel isoelectric focusing.

[‡]To whom reprint requests should be addressed.

tion of the technique described by Sambrook *et al.* (10). The 27 individual donors were of the following genotypes: *1+1+* (two persons), *1−1−* (one person), *1+1−* (two persons), *2+2+* (two persons), *2+2−* (one person), *1+2+* (one person), *1+2−* (one person), *2+7+* (two persons), *2−7+* (one person), *1+7+* (one person), *1+7−* (two persons), *1−7−* (two persons), *1+3+* (five persons), *2+3+* (one person), *1+3−* (one person), *2+3−* (one person), and *1−3−* (one person).

METHODS

The procedures used in this study are summarized in Fig. 1. Briefly, 1 μg of RNA extracted from the cells of each individual was reverse transcribed in 20 mM Tris·HCl/50 mM KCl/2.5 mM $MgCl_2$ buffer, pH 8.4, containing 0.1 mg of nuclease-free bovine serum albumin per ml, for 70 min at 39°C with 200 units of RNase H-reverse transcriptase (Life Technologies, Gaithersburg, MD) using the 18-mer of oligo(dT). An aliquot (1/5th) of the reaction mixture was used for the subsequent PCR. PCR was performed as described (11) with a pair of primers (P1 and P2) whose sequences were situated just 5′ and 3′ to the coding region. The resulting amplicon size was 1760 bp. Sequence analyses of DNAs containing the full open reading frame amplified from mRNA of the individuals whose types were already known were performed by the so-called double-stranded DNA cycle sequencing system method carried out according to the manufacturer's instructions (Life Technologies). Nine types of 19- or 20-mer primers (data not shown) and one 22-mer primer (P2), which was also used for amplification of the 1760-bp fragment, were used as sequencing primers. The primers are arranged at intervals of 300–350 bp. Each DNA was sequenced in both directions.

For determining the haplotype of each gene, amplification was performed with a pair of primers designated P3 (5′-CGATGGATCCGCCAGCCAAGTCCGCCGCTC-3′) and P4 (5′-CGATGGATCCGGACGTACCACATCAGGCCT-3′). These oligonucleotides were designed to include the 6-nucleotide 5′ extensions of the consensus sequence for *Bam*HI restriction endonucleases (underlined in the above sequences) plus four additional nucleotides (boldface in the above sequences). The conditions for these amplifications were the same as those mentioned above except that 25 cycles of the sequential incubation were used in an effort to prevent heteroduplex molecules during the later cycles of amplification (12). After amplification, the PCR products were digested by *Bam*HI and electrophoresed on a 1% agarose gel, from which the appropriate band was extracted. The purified products were ligated to the *Bam*HI site in a pGEM-3 blue vector (Promega) with bacteriophage T4 DNA ligase. To determine the haplotypes of the cloned DNAs, plasmid DNA obtained from each colony of *Escherichia coli* was digested either by a combination of *Bam*HI and *Bgl* II or of *Bam*HI and *Nla* III and subjected to electrophoresis on 1% agarose gel for the former and on 5% polyacrylamide gel for the latter digestions.

To detect polymorphisms in the PCR products amplified by primers P1 and P2, products were digested by either *Bgl* II or *Nla* III and subjected to electrophoresis on 1% agarose gel for the former and on 5% polyacrylamide gel for the latter digestions.

RESULTS

Sequence Analyses of the Four Common Types. To determine the base substitutions, mRNAs from individuals who were either homozygous for the *PGM1*1+*, *PGM1*1−*, or *PGM1*2+* alleles or heterozygous for *PGM1*1+/PGM1*1−*, *PGM1*2+/PGM1*2−*, or *PGM1*1+/PGM1*2+* were amplified by reverse transcriptase/PCR (Fig. 1*A*). Direct sequencing of the 1760-bp amplified products using the cycle sequencing method (Fig. 1*B*) identified two types of base substitutions. As shown in Fig. 2*A*, the individuals homozygous for *PGM1*1+* were characterized by C at nucleotide 723, whereas the individuals homozygous for *PGM1*2+* exhibited T at the same position. The heterozygous individual for *PGM1*1+/PGM1*2+* was found to possess either C or T at position 723. A homozygote for *PGM1*1−* and heterozygotes for *PGM1*1+/PGM1*1−* also had C at this position, whereas a *PGM1*2+/PGM1*2−* individual possessed T at the same position (data not shown). On the other hand, as shown in Fig. 2*B*, the individual whose type was *PGM1*1+* had T at residue 1320 (A in the photograph, since the antisense sequences are shown in the sequence gel) and the individual whose type was *PGM1*1−* had C at the same position. The heterozygous individuals for *PGM1*1+/PGM1*1−* exhibited T and C at position 1320. The individuals whose types were *PGM1*2+* and *PGM1*1+/PGM1*2+* possessed a T at position 1320, while the heterozygote for *PGM1*2+/PGM1*2−* possessed both C and T at this position (data not shown).

Thus, the data from the sequencing analyses demonstrated that the change from type 1 (*PGM1*1+* and *PGM1*1−*) to type 2 (*PGM1*2+* and *PGM1*2−*) was caused by a C to T transition at residue 723. Furthermore, a T to C transition at residue 1320 was responsible for the conversion of type + (*PGM1*1+* and *PGM1*2+*) to type − (*PGM1*1−* and *PGM1*2−*). The base substitution at residue 723 created a *Bgl* II site in the 1760-bp PCR product, and the base substitution at residue 1320 created a *Nla* III site. As shown in the agarose gel (Fig. 3*A*), *Bgl* II digestion of the PCR products yielded either a 1760-bp uncut fragment for allele type 1 or a 1059- and a 701-bp fragment for allele type 2. As revealed by PAGE (Fig. 3*B*), *Nla* III digestion of the PCR products yielded either a 600-bp fragment for allele type + or a 344- and a 256-bp fragment for allele type − in addition to fragments of 478, 198, 149, 110, and 104 bp, which are shown in Fig. 3*B*, as well as fragments of 48, 41, and 16 (2) bp, which are not

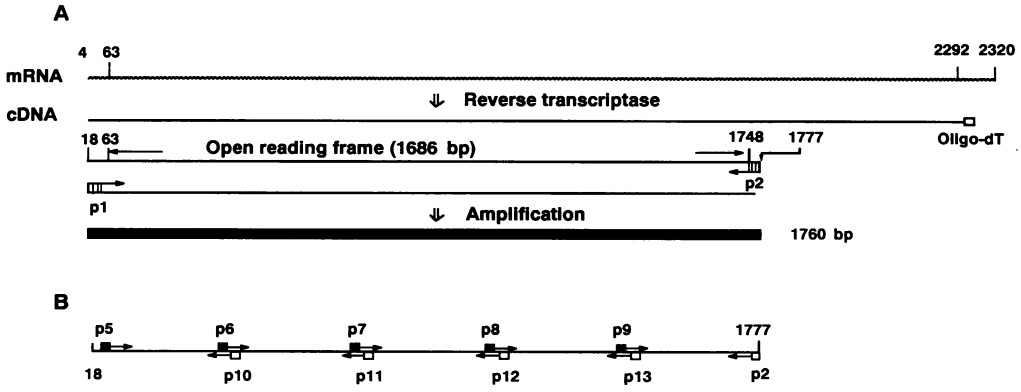

FIG. 1. Schematic diagram of procedures for sequencing mRNA of *PGM1*. The numbering system of Whitehouse *et al.* (8) is used. (*A*) Amplification of mRNA of *PGM1* gene. (*B*) Primers for the cycle sequencing method. Arrows with boxes indicate position and orientation.

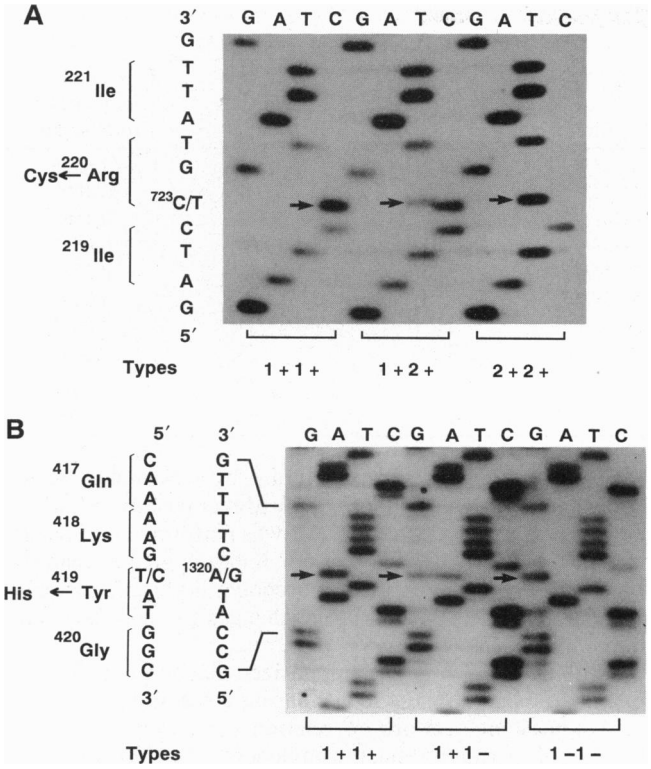

FIG. 2. Direct sequencing of cDNA of the common *PGM1* types. Genotype of each individual is shown below. Arrows indicate positions of base substitutions. Numbering system of amino acids of Whitehouse *et al.* (8) is used. (*A*) Sequences of cDNA from the individuals who show the type 1 alleles and the type 2 alleles. (*B*) Sequences from individuals who show the type + alleles and the type − alleles. Sequence gel shows the antisense sequences from cDNA. The sense sequence is written adjacent to the sequence from the gel.

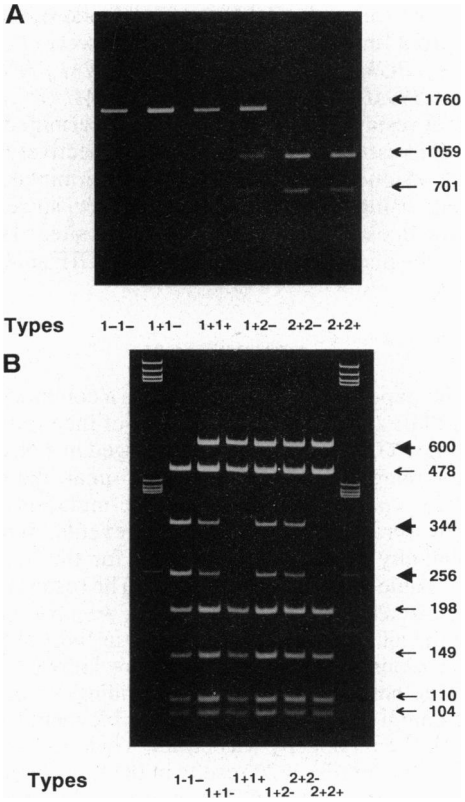

FIG. 3. Detection of *Bgl* II and *Nla* III restriction fragment length polymorphisms after reverse transcriptase/PCR amplification. Genotype is shown under each lane. Sizes of fragments produced by digestions are shown on the right. (*A*) Reverse transcriptase/PCR amplified fragments (1760 bp) were treated by *Bgl* II and analyzed on a 1% agarose gel. (*B*) Amplified fragments (1760 bp) were treated by *Nla* III and analyzed on 5% polyacrylamide gel. Outside lanes contain *Hae* III-digested ColE1 as a size marker. Boldface arrows point to bands that show variations and thin arrows point to constant bands.

shown for both alleles. These sequencing data permit the inference that amino acid 220 of type 1 was Arg and that of type 2 was Cys, and that the Tyr at amino acid 419 in type + was replaced by a His in type −. Preliminary data concerning these amino acid substitutions have been presented elsewhere (13), in which the amino acid sequences were numbered from the initial Met.

Sequence Analyses of the Four Rarer Types. Sequence analyses were carried out for the DNA amplified from mRNA of the individuals whose genotypes were *PGM1*2+/PGM1*7+* (two persons), *PGM1*2−/PGM1*7+* (one person), *PGM1*1+/PGM1*7+* (one person), *PGM1*1+/PGM1*7−* (two persons), or *PGM1*1−/PGM1*7−* (two persons). All eight individuals, who as a group exhibited five different phenotypes, were characterized by both A and T at residue 265. Thus, when the DNA from a *PGM1*1+/PGM1*7+* individual was examined, it had the same sequence as the *PGM1*1+* genotype, except that *PGM1*1+/PGM1*7+* was found to possess either A or T at residue 265. The individuals who were homozygous for type + (e.g., *PGM1*2+/PGM1*7+* and *PGM1*1+/PGM1*7+*) showed only T at residue 1320, while type − (e.g., *PGM1*1−/PGM1*7−*) showed C at the same position. The heterozygotes for type + and type − (*PGM1*2−/PGM1*7+*, *PGM1*1+/PGM1*7−*), of course, possessed either C or T at residue 1320. Moreover, the individuals who differed in their type 2 allele (*PGM1*2+/PGM1*7+*, *PGM1*2−/PGM1*7+*) showed either C or T at residue 723. The presence of either type 2 or type − alleles was also confirmed by restriction fragment length polymorphism analysis of the PCR products as mentioned before. The results revealed that an A to T transversion at residue 265 had occurred in the type 7 alleles. These sequencing data thus suggest that the Lys residue at amino acid 67 of the common types (types 1 and 2) was replaced by Met in types 7 (*PGM1*7+* and *PGM1*7−*).

Sequence analyses were also carried out for nine individuals heterozygous for either *PGM1*3+* or *PGM1*3−* and one of the common alleles [*PGM1*1+/PGM1*3+* (five persons), *PGM1*2+/PGM1*3+* (one person), *PGM1*1+/PGM1*3−* (one person), *PGM1*2+/PGM1*3−* (one person), *PGM1*1−/PGM1*3−* (one person)]. All of these individuals showed an A to T substitution at residue 265 and a C to T substitution at residue 723. In particular, when the heterozygotes for *PGM1*2+/PGM1*3+* and *PGM1*2+/PGM1*3−* were examined, only T was observed at residue 723. The individuals who were homozygous for type + (e.g., *PGM1*1+/PGM1*3+*, *PGM1*2+/PGM1*3+*) had T at residue 1320 and the homozygous individual for types − (e.g., *PGM1*1−/PGM1*3−*) had C at the same position. The heterozygotes for type + and type − (*PGM1*1+/PGM1*3−* and *PGM1*2+/PGM1*3−*) possessed either C or T at position 1320. These sequence data demonstrated that type 3 (*PGM1*3+* and *PGM1*3−*) is characterized by Met at residue 67 and Cys at residue 220, but those possessing the *PGM1*3+* allele had Tyr at residue 419, whereas those with *PGM1*3−* had His. A comparison of the data on the four common alleles that were detected as heterozygous with that on the four rarer alleles demonstrated that the base substitutions detected in the rare alleles are consistent with those determined by analysis of the common alleles.

Haplotypes for each allele were examined by using PCR samples from individuals whose types were *PGM1*1+/PGM1*7−*, *PGM1*2+/PGM1*7+*, *PGM1*1+/PGM1*3+*, *PGM1*1−/PGM1*3−*, or *PGM1*2+/PGM1*3−*. The substitutions at residues 723 and 1320 were determined by *Bgl* II and *Nla* III restriction site analysis, respectively. The sequence at residue 265, however, was determined by direct sequencing, using P10 as a sequence primer, since this base substitution does not affect any restriction sites. The results confirmed the phenotypes observed by PAGIF analysis (data not shown).

DISCUSSION

In an earlier paper (7), we suggested from a combination of the results of SGE and of PAGIF that eight of the more common alleles of the *PGM1* locus could be arranged in a phylogeny in which, assuming the *PGM1*1+* to be primal, the remaining seven alleles could be derived by three mutations and four subsequent intragenic recombinational events. With the recent availability of a cDNA sequence for this locus (8), it became possible to test this hypothesis. The results of this test are summarized in Fig. 4. For continuity, we have retained the format of the allele phylogeny published in 1982, although the relative positions of the mutations are now known not to be as postulated at that time. The nucleotide findings reported in this paper are completely congruent with the biochemical findings upon which the phylogeny was based. Thus, the substitution of T by C at nucleotide 1320 results in the replacement of Tyr by His at position 419 in the PGM1 polypeptide, which creates the type − variant with the higher pI. The substitution of C by T at nucleotide 723 results in the replacement of Arg by Cys at position 220 in the polypeptide, which results at the pH at which electrophoresis was performed (pH 7.4) in a "fast" variant (type 2). The substitution of A by T at nucleotide 265 results in the replacement of Lys by Met at position 67, which at the pH at which electrophoresis was performed also results in a fast variant, but one whose mobility is greater than the preceding one (type 7). Four subsequent intragenic recombinations reshuffled these primary changes to yield four additional phenotypic variants.

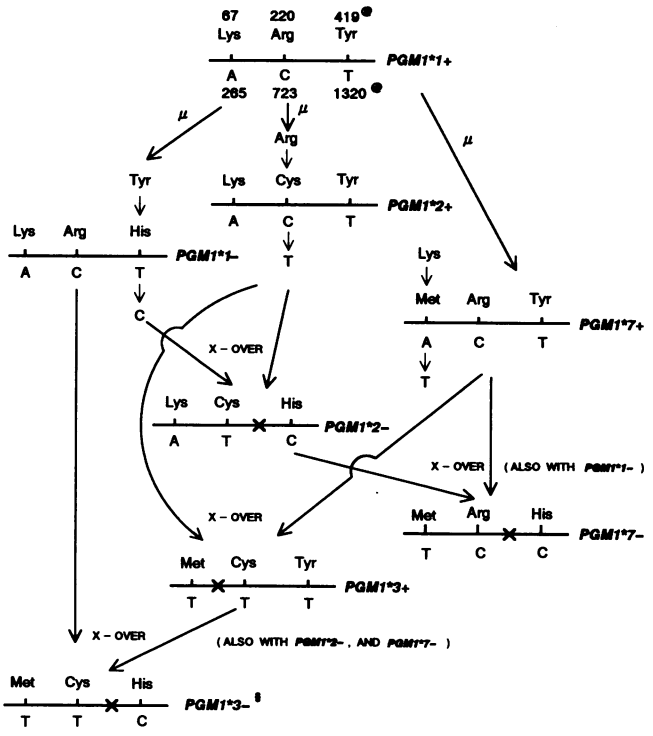

FIG. 4. Phylogeny relating eight alleles in the *PGM1* system. Positions and types of base substitutions and amino acid substitutions derived from the base sequence are presented. μ indicates origin through mutation. Position of the postulated crossover is in each instance indicated by ×. @, The numbering system of Whitehouse *et al.* (8) is used; §, *PGM1*3−* is produced directly by crossover between *PGM1*2−* and *PGM1*7+*.

Table 1. Allele frequencies at the *PGM1* locus in a sample of Japanese drawn from Hiroshima and Nagasaki

Allele	Nucleotide substitutions	Estimated no. in total sample	Estimated allele frequency in total sample
*PGM1*1+*	ACT	19,897	0.6525
*PGM1*1−*	ACC	3,348	0.1098
*PGM1*2+*	ATT	4,766	0.1563
*PGM1*2−*	ATC	2,003	0.0657
*PGM1*7+*	TCT	372	0.0122
*PGM1*7−*	TCC	15	0.0005
*PGM1*3+*	TTT	37	0.0012
*PGM1*3−*	TTC	9	0.0003
Other	—	46	0.0015
		30,493	1.0000

It should at this point be emphasized that although first the phenotypic and now the molecular data permit construction of a consistent network of relationships, they do not by themselves permit rooting the phylogeny. We have elected to root the phylogeny in the *PGM1*1+* allele because this is worldwide the most common and hence presumably the oldest allele and also appears to be identical to the *PGM1* of other higher primates (*Pan, Gorilla*, and *Pongo*) (6). Conversely, the alleles treated as the later developments in the phylogeny are less common and more restricted in their distribution, with the *PGM1*3+* and *-3−* and *PGM1*7+* and *-7−* alleles being largely confined to Asian–Pacific populations. (Now that a cDNA probe is available, it will be possible to determine whether the occasional reports of PGM1 3 and PGM1 7 phenotypes outside this region result from the mutations identified in this study or from other alleles with a similar phenotype.)

Given the verification of the allele relationships depicted in Fig. 4, it becomes of considerable genetic interest to examine the amount of intragenic genetic disequilibrium the postulated sequence of events has created at this locus. The data on PGM1 at our disposal results from a survey of 30,493 alleles for PGM electrophoretic types conducted in the course of studies on the potential genetic effects of atomic bombs (14). Subsets of this total sample have been examined to define the frequency of the two isoelectric focusing types within each of the electrophoretic types (7). The multiplicative combination of the results of these two examinations enables us to calculate the numbers and proportions in the total Hiroshima–Nagasaki sample of each of the eight genotypes depicted in Fig. 4. The results of this calculation are shown in Table 1. The term "other" in Table 1 encompasses 13 different variants defined by multiple cross comparisons (3, 14, 15). Locus linkage disequilibrium for these data has been analyzed as suggested by Weir (16). We let N1, N2, and N3 denote the nucleotide substitutions (left to right) and p denote the frequency of the two nucleotides at each substitution site. Then,

$$\text{N1: } p(\text{A}) = 0.9858 \quad p(\text{T}) = 0.0142$$

$$\text{N2: } p(\text{C}) = 0.7762 \quad p(\text{T}) = 0.2238$$

$$\text{N3: } p(\text{T}) = 0.8235 \quad p(\text{C}) = 0.1765.$$

Table 2. Linkage disequilibrium at the *PGM1* locus

Definition of comparison	D_{AB}	min(D_{AB})	max(D_{AB})	var(D_{AB})	χ^2	P
A = N1(A) B = N2(C)	−0.0017	−0.7652	0.0110	7.99×10^{-8}	36.18	$<10^{-8}$
A = N1(A) B = N3(T)	−0.0018	−0.8118	0.0117	6.68×10^{-8}	48.48	$<10^{-11}$
A = N2(C) B = N3(T)	0.0265	−0.6392	0.1370	8.29×10^{-7}	847.10	∞

For any two substitution sites, with alleles A and a and B and b, respectively, the gametic disequilibrium, D_{AB}, is defined as $D_{AB} = p_{AB} - p_A p_B$, where p_{AB} is the haplotype frequency. The minimum and maximum values possible for D_{AB} depend on the allele frequencies at each locus, as follows: $-p_A p_B \leq D_{AB} \leq \min(p_a p_B, p_A p_b)$. The variance under the null hypothesis that $D_{AB} = 0$ can be estimated by using $\text{var}(D_{AB}) = p_A p_a p_B p_b / n$. To test the null hypothesis that $D_{AB} = 0$, the statistic $X^2 = D_{AB}^2/\text{var}(D_{AB})$ can be used, which is distributed approximately as a χ^2 with 1 degree of freedom. The results of this analysis are given in Table 2.

For three substitution sites, 1, 2, and 3, with alleles A and a, B and b, and C and c, respectively, the gametic disequilibrium D_{ABC} is defined as $D_{ABC} = p_{ABC} - p_A D_{BC} - p_B D_{AC} - p_C D_{AB} - p_A p_B p_C$, where p is the allele frequency and D is the appropriate gametic disequilibrium. To test the null hypothesis that $D_{ABC} = 0$, the statistic $X^2 = D_{ABC}^2/\text{var}(D_{ABC})$ can be used. It is distributed approximately as a χ^2 with 1 degree of freedom. Setting A = N1(A), B = N2(C), C = N3(T), then $D_{ABC} = -3.657 \times 10^{-5}$, $\text{var}(D_{ABC}) = 5.198 \times 10^{-9}$, and $X^2 = 0.2572$. [The very rare (other) variants listed in Table 1 cannot enter into this calculation because the nucleotide substitutions that distinguish them have not yet been established.]

There are two noteworthy results of this analysis. First, all the disequilibria are relatively small albeit, by virtue of the relatively large numbers involved, significant. Thus, we define a statistic D'_{AB} as $D'_{AB} = D_{AB}/p_A p_B$, where $D_{AB} < 0$ and $D'_{AB} = D_{AB}/\min(p_a p_B, p_A p_b)$ when $D_{AB} > 0$. Unlike D_{AB}, this statistic has a range from −1.0 to +1.0; this property permits the direct comparison of the various disequilibria. From the values given in Tables 1 and 2, we obtain $D'_{(1)} = -0.0022$, $D'_{(2)} = -0.0022$, and $D'_{(3)} = 0.1934$, where the subscripts correspond to the rows in Table 2. Two of the disequilibria are negligible; the other is modest. This approach to equilibrium is also reflected in the small value of D_{ABC} as defined above. Second, we note that $D'_{(3)}$ is larger than $D'_{(1)}$ or $D'_{(2)}$, despite the apparent reasonableness of the postulate that the mutation resulting in *PGM1*7+*, which created $D'_{(1)}$ and $D'_{(2)}$, arose at some considerable interval after the two mutations creating $D'_{(3)}$.

We note that the substitution of C for T at residue 723 created a *Bgl* II restriction enzyme cut site in the 1760-bp PCR product, and the substitution of T for C at residue 1320 created an *Nla* III site. These restriction sites create the possibility of rapid typing from a DNA sample of some of the more common alleles of the *PGM1* locus, a development of possible use in situations in which only DNA is available, as in forensic medicine.

We are grateful to Mr. H. Omine, Mr. Y. Kimura, Ms. J. Kaneko, and Ms. A. Miura for their technical contributions throughout this investigation. We would also like to thank Dr. J. Asakawa and Mr. S. Kaneoka for synthesis of the primers. We thank Drs. C. Satoh and M. Kodaira for many helpful discussions. We are much indebted to Ms. M. Kamouchi for collection of blood samples. Dr. Martha Haviland has been of invaluable assistance in the disequilibrium analysis. Finally, we thank Dr. D. A. Hopkinson for making the cDNA sequence of *PGM1** available to us 4 months in advance of publication. This publication is based on a manuscript (RPs 4-75 and 7-85 and MS 21-93) resulting from research performed at the Radiation Effects Research Foundation (RERF), Hiroshima and Nagasaki, Japan. RERF is a private foundation funded equally by the Japanese Ministry of Health and Welfare and the U.S. Department of Energy through the National Academy of Sciences. J.V.N. acknowledges the support of DOE Grant ER-60533.

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