Hum Genet (1993) 92:470–476

human genetics

Frequency of glucose-6-phosphate dehydrogenase (G6PD) mutations in Chinese, Filipinos, and Laotians from Hawaii

Y. Edward Hsia[1, 2], Faye Miyakawa[1], John Baltazar[1], Nathan S.P. Ching[1], Juliet Yuen[2], Beryl Westwood[3], Ernest Beutler[3]

[1] Department of Genetics and Pediatrics, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96822, USA
[2] Kapiolani Medical Center for Women and Children, 1919 Punahou Street, Honolulu, HI 96826, USA
[3] Scripps Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA

Received: 13 January 1993 / Revised: 30 March 1993

Abstract. In a Hawaii Hereditary Anemia Screening Project, 4,984 participants were tested for glucose-6-phosphate dehydrogenase (G6PD) deficiency by a filter paper blood spot fluorescence test. Abnormal samples and suspected heterozygotes were checked by quantitative G6PD assay (normal 4.5 to 14 units/g Hb). G6PD was deficient (< 1.5 units/g Hb) in 188 of 2,155 males; 7 other males had low activity (1.5 to 2.8 units/g Hb). The gene frequency, estimated from males after excluding referred and related cases, was 0.037 for Chinese, 0.134 for Filipinos, and 0.203 for Laotians. Among 2,829 females tested, family data showed 111 females were obliged to be at least heterozygous, regardless of G6PD activity, and 43 others had low G6PD activity. Most heterozygotes probably remained undetected by G6PD screening. In 28 females, activity was under 10%; in another 9 females, activity was < 1.5 units/g Hb. Since only 25 homozygotes would be predicted, this apparent excess of females with deficient activity could be due to unequal X-inactivation in some heterozygotes. DNA analysis by polymerase chain reaction amplification and special analytic procedures revealed 10 different missense mutations in 75 males. The nucleotide 835 A→T and 1360 C→T transitions were first detected in this Hawaiian Project; we found that the nucleotide 1360 mutation was the most common cause of G6PD deficiency in Filipinos. This is the first report of G6PD screening and analysis of molecular G6PD mutations in Filipino and Laotian populations.

Introduction

Sex-linked recessive glucose-6-phosphate dehydrogenase (G6PD) deficiency is estimated to affect over 100,000,000 human males worldwide; most are of African, Middle Eastern, Asian, or Southeast Asian ancestry (Beutler 1991).

Over 400 variants have been recorded, differing in severity, clinical expression, and biochemical properties (Beutler and Yoshida 1988; Beutler 1990). Among class 3 types with 10%–60% enzyme activity (Yoshida et al. 1971), relatively mild variants, such as the A^- type common in Africans, render red cells susceptible to oxidative damage. In Mediterraneans, Southern Chinese, and Southeast Asians, class 2 moderately severe variants with < 10% activity are important causes of neonatal jaundice (Beutler 1990, 1991), and of drug-induced or fever-induced hemolytic anemias (Missiou-Tsagaraki 1992; Beutler 1991). The more severe, class 1 variants, which cause debilitating chronic hemolytic anemia, are uncommon; the most severe variants, which also cause chronic granulomatous disease, are very rare.

Gene frequency and prevalence data have been reported in many studies from Chinese populations (Du et al. 1988; Panich 1986), but few studies have reported on G6PD deficiencies in Filipinos or Laotians (Motulsky et al. 1964; Nitowsky et al. 1965; Fernandez and Fairbanks 1968; Yoshida et al. 1970; Sicard et al. 1978; Panich 1986).

The G6PD allele has been sequenced (Persico et al. 1986), and a growing number of human mutations have been described, virtually all of which are missense mutations. Specific mutations have been identified for many variants; some apparently different biochemical variants have been found to have the same mutation (Beutler 1992).

In the Hereditary Anemia Screening Project in Hawaii, we have screened families for G6PD deficiency (Hsia 1991), as well as for the α- and β-thalassemias (Hsia et al. 1988). We have compared the qualitative and quantitative G6PD data from these populations, and estimated gene frequencies for deficient G6PD variants of Chinese, Filipino, and Laotian subpopulations in Hawaii (Hsia et al. 1992). DNA has been analyzed for G6PD mutations in selected males (Beutler et al. 1992b).

Correspondence to: Y. E. Hsia, Department of Genetics and Pediatrics, John A. Burns School of Medicine, Kapiolani Med. Ctr. for Women and Children, 1319 Punahou St., Honolulu, HI 96826, USA

Table 1. Results of 4,439 qualitative[a] and 545 quantitative glucose-6-phosphate dehydrogenase (G6PD) assays

G6PD Activity	Normal		Low		Deficient		Total tested
	+++/+++	≥ 4.5 (units/g Hb)	+/++	1.5–4.4 (units/g Hb)	–	< 1.5 (units/g Hb)	
Males	1,854	106		7	8	180	2,155
Females[b]	2,546	110	9	34		19	2,718
Heterozygotes[c]		50	22	21		18	111
Total	4,666		93		225		4,984

[a] Qualitative activity was scored using a standard fluorescence test with (++++) for full fluorescence to (–) for none

[b] Only females who were not obliged to be heterozygous by family history

[c] Females who were obliged to be heterozygous by family history, regardless of G6PD activity

Table 2. Quantitative G6PD activity ranges in 376 subjects over 6 years, and 56 infants under 2 months of age

G6PD activity	Subpopulation					
	Older subjects			Young infants		
	Number	Mean	1 SD	Number	Mean	1 SD
Normal males	80	6.63	1.47	11	10.57	3.22
Low activity males	6	1.90	0.46	1	2.80	
Deficient males	110	0.42	0.35	25	0.31	0.33
"Normal" females	104	5.59	4.00	9	8.54	3.63
Obligate heterozygotes	63	4.88	4.06	8	4.51	3.63
Positive family history	13	5.66	3.52	2	11.80	

Materials and methods

In Hawaii, from 1984 to 1992, over 6,000 individuals were screened for the thalassemias, and from 1986 to 1992, 4,984 were screened for G6PD deficiency, under an institutionally approved protocol (Table 1). Families were recruited by public announcements, by a Laotian outreach worker, at community health fairs, and by physician referrals of suspected thalassemic heterozygotes and their relatives. A few families were referred for screening because a boy was diagnosed to have G6PD deficiency. Blood was collected by collaborating clinical laboratories, and sent to the central laboratory on wet ice. No sample was taken during a hemolytic crisis or immediately after a transfusion.

Qualitative G6PD activity was assayed on eluates from dried filter paper blood spots, using a standard fluorescence test, and scored subjectively by intensity of fluorescence (Beutler 1966). Quantitative activity was assayed by a kit (Sigma Chemical, St Louis, Mo.), according to the manufacturer's instructions. This was done when samples had less than full fluorescence (++++) and when females were possible or obligate heterozygotes. A female was designated to be at least heterozygous when her father or son was found to have subnormal G6PD, or her daughter had low activity but her husband had normal results.

Based on quantitative results in adult males (Table 2), we determined that normal activity ranged from 4.5 to 17 units/g Hb (mean 6.63 units ± 1.63); but subnormal results were distributed in a "deficient" group with 1.5 units activity or less (mostly class 2, some class 3). Only a few samples were in a "low" group with over 1.5 units but under 3.0 units activity (class 3). A male with deficient or low activity was diagnosed to be hemizygous for a G6PD mutation; a female with low activity was diagnosed to be heterozygous; and the deficient activity of one female was attributed to her being either heterozygous or homozygous for G6PD deficiency.

For calculating gene frequencies, to allow for bias of ascertainment, we excluded those referred for G6PD deficiency and counted only one related male from each family. One G6PD deficient male who had partial Chinese, and 15 who had partial Filipino ancestry, were not included when estimating ethnic frequencies.

DNA was extracted from blood of subjects over age 6 years to analyze for α-globin deletions (Hsia et al. 1988). DNA from 81 selected males with subnormal G6PD was analyzed, using a sequential strategy to identify mutations known to occur in these sampled populations (Fig. 1; Beutler et al. 1991a).

Samples were tested successively by the following steps, until a mutation was identified. First, polymerase chain reaction (PCR) was used to amplify exon 9 of the G6PD locus using previously described primers (Fig. 1; Beutler et al. 1991a). The nucleotide (nt) 871 G→A mutation was detected by allele-specific oligonucleotide hybridization (ASOH), and the nt 1003 G→A mutation, by direct sequencing. The nt 871 G6PD^{Jammu} from India (Beutler 1976), or G6PD$^{Viang\ Chang}$ from Laos differ by nt 1311 C/T and intron 11 *Hla*III polymorphisms; these were distinguished by haplotype analysis (Beutler et al. 1992c).

Exons 11 and 12 were amplified by PCR using previously described methods (Beutler et al. 1991a). 5′-TGGATCAGCAAGACACTCT and 3′-ACTCCCACCTCGATGGAGTA primers were use to generate a 325-bp fragment. ASOH was used to detect the known G→A substitutions at nt 1376 and nt 1388, equivocal results were checked by direct sequencing. After the nt 1360 C→T mutation was found to be common, ASOH was also used on the same amplified fragment to detect this. Since this mutation destroys an *Fsp*I site, equivocal results were confirmed by restriction digest analysis using this enzyme. 5′-TGGCATCAGCAAGACACTCT and 3′-GGACGTATGGACACCCGATA primers were used to amplify a 146-bp fragment. *Fsp*I digestion splits normal samples into 102-bp and 44-bp subfragments.

The remaining samples were examined for the nt 835 A→T mutation. The 3′-CCGGGTAGACACTGCTCCGT and mismatched 5′-GCCATGGAGAAGCCCGACTC primer were used to amplify

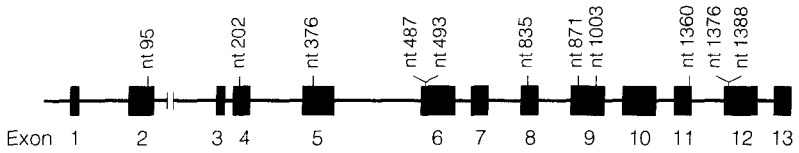

Fig. 1. The G6PD gene on Xq28, showing the ten mutations found in the Hawaii study (Beutler et al. 1991a)

a 110-bp fragment. Since this mutation creates an *MnlI* site, the PCR product was digested with *MnlI*, producing 79-bp and 31-bp subfragments when samples had the nt 835 mutation.

Exons 6, 7, and 10 were independently amplified and screened for mutations using single-strand conformational polymorphism (SSCP) analysis (Orita et al. 1989). Samples with abnormal results were sequenced. The mutations at nt 487 G→A and at nt 493 A→G, in exon 6, generated *AluI* and *Sau*961 sites, respectively.

These mutations were confirmed by amplification of a 70-bp fragment using 5′-GGTTCAAGGGGGTAACGCAG and 3′-AGTAGCACCTCTTCGGGAAG primers. Upon digestion with the appropriate enzyme, creation of an *AluI* site by the nt 487 mutation resulted in bands of 20-bp, 31-bp, and 19-bp. Creation of a *Sau*961 site by the nt 493 mutation resulted in bands of 46-bp and 24-bp.

Samples from the one subject with African ancestry who had the nt 1360 mutation and from two other subjects with African ancestry who had low G6PD activity, were tested for the nt 202 C→A and nt 376 A→G mutations of the African A⁻ variant by *Nla*III and *Fok*I digests (Hirono and Beutler 1988).

Finally, a 104-bp fragment of exon 2 was amplified using a mismatched primer, 5′-CTTCCATCAGTCGGATACGC and 3′-GTTAGAATTTTCGGTCCTTC (Beutler et al. 1991a) to create a *HhaI* site if the nt 95 A→G mutation was present. This mutation was detected when digestion with *HhaI* gave bands of 82-bp and 22-bp. All restriction digests were carried out according to the manufacturers' recommended protocols.

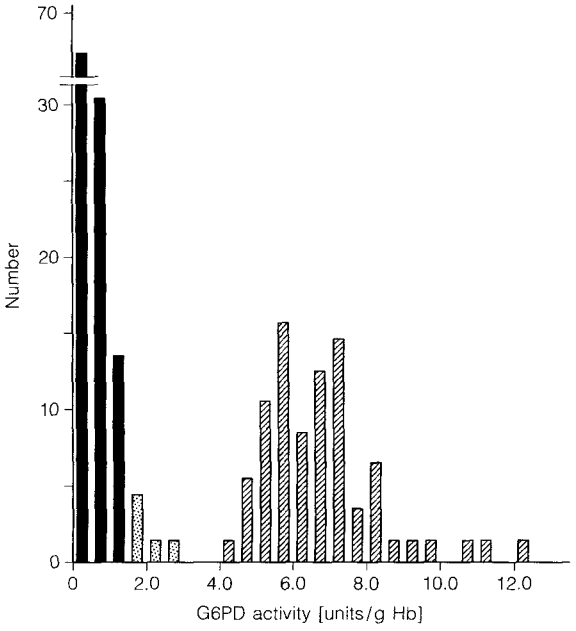

Fig. 2. G6PD activity in males over age 6. Deficient: ■ ≤ 1.5; low: ■ 1.5–4.4; normal: ▩ ≥ 4.4

Results

Screening was completed in 4,984 samples from all ages, and quantitative assays were done in 545 (Table 1, Fig. 2). Quantitative assays in 250 samples with full fluorescence (++++) were normal in all but 11; the lowest activity with (++++) was 2.6 units. Quantitative assays in 35 samples with (+++) were normal in all but 8; the lowest was 2.3 units. All 14 samples with (++) were assayed; activity ranged from 1.4 to 4.2 units. All 22 samples with (+) were assayed; activity ranged from 0.3 to 4.3 units, and 7 were deficient. All but 8 samples with no fluorescence (–) were assayed; one female with (–) had activity of 4.1 units.

Quantitative assays were done in 293 of 2,155 males who had qualitative testing, including 187 of 195 with (–) or (+) qualitative results. One borderline male who had (+++) and 4.4 units activity was considered normal; none of the others had > 2.8 units activity. G6PD activity was deficient in 188 males (120 were class 2, 68 class 3) and low in 7 (Table 1, Fig. 2). Some samples were not tested until 4 or more days after collection, due to delays in transfer to our central laboratory, but this was associated with no noticeable lowering of either qualitative or quantitative results.

Based on findings in relatives, 111 females were obliged to be at least heterozygous for a deficient variant, 89 of these had quantitative assays (Table 1, Fig. 3). Among these obligate heterozygotes, activity was normal in 50, low in 21, and deficient in 18 (11 of these had < 10% activity), 7 other females with affected relatives also

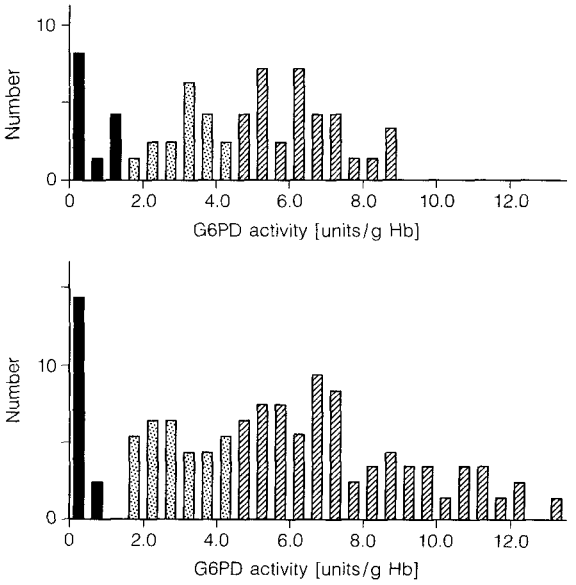

Fig. 3. G6PD activity in obligate heterozygotes *(upper histogram)* and other females *(lower histogram)* over age 6

had low activity. Of 2,656 other females with (++++) or (+++) results, 110 had quantitative assays; of 62 with (++), (+), or (–) results, 53 had quantitative assays. Results were low in a total of 55 females and deficient in a total of 37 females (28 of these had < 10% activity). The

Table 3. Gene frequencies for G6PD deficiency by race[a]

	Ethnicity		
	Chinese	Filipino	Laotian
Total males	272	783	74
Affected males	10	105	15
G6PD deficiency	0.037	0.135	0.202

[a] Referred or related cases and those with mixed ancestry were excluded

family history did not prove or exclude homozygosity in any of the 37 females with deficient activity.

The means and standard deviations of quantitative G6PD were calculated separately for infants and for those over age 6 years, in males who were normal, low, or deficient, in females obliged to be heterozygous, and in other females (Table 2). Excluded from these calculations were two males, three obligate heterozygotes, and four other females who had excessive activities (27–69.3 units).

After allowing for bias of ascertainment, 157 of 1,983 G6PD alleles in males were deficient, and 6 were low; three of the four African Americans with abnormal G6PD had low results.

Calculated gene frequencies for G6PD-deficient alleles in Chinese, Filipinos, and Laotians are in Table 3.

DNA was analyzed on samples from 75 males with low or deficient G6PD, including three brother-pairs. Two transversion and eight transition missense mutations at the G6PD locus were found (Table 4, Fig. 1), including 2 reported for the first time from Hawaii (Beutler et al. 1992b). No mutation was detected in 6 other samples that had insufficient DNA for completing the sequential analysis. The 1360 mutation was common in Filipinos but was also found in one Laotian and one African American who was born in Florida and was unlikely to have any Southeast Asian ancestry.

We found the nt 1360 mutation had well under 10% G6PD activity. The nt 95, nt 1003, and nt 1376 mutations also had < 10% activity; the nt 487, nt 493, nt 871, and nt 1388 mutations had > 10% activity. The A^- and nt 835 mutations had > 20% G6PD activity.

Quantitative G6PD activity had been determined in 68 of the 75 subjects who had DNA analyses. From these, the mean and standard deviation were calculated for each mu-

Table 4. G6PD DNA mutations in 72 chromosomes by race

G6PD mutation	Chinese	Filipino	Laotian	African American	Japanese	Total
95 A→G Arg→Cys	3					3
202 G→A Val→Met 376 A→G Asn→Asp				2[a]		2
487 G→A Gly→Ser			2			2
493 A→G Asn→Asp		4				4
835 A→T Thr→Ser	1					1
871 G→A Val→Met		6	5			11
1003 G→A Ala→Thr		8				8
1360 C→T Arg→Cys		35	1	1		37
1376 G→T Arg→Leu					1	1
1388 G→A Arg→His	2		1			3
Total	6	53	9	3	1	72

[a] These samples had the nt 376 mutation of the A+ variant and the common nt 202 mutation of the A– subvariant

Table 5. Means and standard deviations of G6PD activity in 68 mutant samples, including three brother-pairs

G6PD mutation	Name	Class	Number	Mean	1 SD
95 A→G Arg→Cys	Gao He	3	3	0.47	0.06
202 G→A Val→Met 376 A→G Asn→Asp	A–	3	2	1.70	
487 G→A Gly→Ser	Mahidol	3	3	0.80	0.10
493 A→G Asn→Asp		3	3	0.90	0.35
835 A→T Thr→Ser		3	1	2.30	
871 G→A Val→Met	Viang Chan	2	12	0.99	0.30
1003 A→G Ala→Thr	Chatham	3	5	0.38	0.16
1360 C→T Arg→Cys		2	35	0.21	0.25
1376 G→T Arg→Leu	Canton[a]	3	1	0.40	
1388 G→A Arg→Hi	Kai Ping	2	3	0.87	0.15

[a] Also Taiwan Hakka, Class2

tation (Table 5). We were unable to obtain additional samples from these subjects for G6PD enzyme electrophoresis or full biochemical characterization.

Discussion

Specificity and sensitivity

In comparing qualitative with quantitative testing, we found qualitative fluorescence was simple and sufficient for identifying moderately severe G6PD variants, although it could have missed some mild variants that had borderline low (class 3) quantitative activity. A (–) or (+) results was always subnormal on quantitative testing, but was sometimes > 1.5 units; a (++) result was likely to have > 1.5 units activity, while a (+++) or (++++) result did not always rule out class 3 deficiency (1.5–3.0 units). Subnormal activities in males were all well below the normal range.

In infants, normal values had a higher range, possibly due to raised nucleated cells, reticulocytes and platelets (Morelli et al. 1981; Table 2). This could have caused more G6PD-deficient infants to have activity > 1.5 units, but only 1 male infant was among the 7 with > 1.5 units activity, while 37 infants had < 1.5 units. Therefore, qualitative testing was unlikely to have missed moderately deficient class 2 G6PD variants in infants. Three of the 7 males with low activity had mutation analysis; all were found to have mild class 3 variants.

Assuming that the normal range was similar in both sexes, females with low activity were diagnosed to be heterozygous. About half the obligate heterozygotes had normal results; therefore, many additional heterozygotes must have been among those with normal test results.

Seven of the 9 samples with exceptionally high results were from children, 4 were associated with leucocytosis (white cell counts over 11.5×10^9/l). Based on Hardy-Weinberg equilibrium for the estimated gene frequencies in each ethnic group, we had expected to find 25 homozygous females, but 37 had deficient class 2 or class 3 activity. This apparent excess of G6PD-deficient females is likely to be due to imbalanced X-inactivation in heterozygotes (Beutler et al. 1962).

Gene frequencies

The gene frequency of G6PD deficiency in Chinese from Hawaii was similar to that reported in other Chinese populations (Panich 1986; Du et al. 1988), but Filipinos and Laotians showed much higher frequencies (Table 3). The large numbers that we tested leave little doubt about the data for Filipinos. The data from Laotians might have been exaggerated by smaller sample size and possible selection bias, but must still be very high.

Over 95% of the Chinese in Hawai are originally from a small region of Guangzhou Province, and over 95% of the Filipinos are from northern Luzon, so the results need not be representative of other regions in these countries. The Laotians were from the northern, central, and southern regions of Laos.

Molecular mutations

Most of the mutations we found in Hawaii (Tables 4, 5) had been reported previously, but with different population distributions. The nt 95 G6PD^{GaoHe} reported in some Chinese from Taiwan (Chiu et al. 1991b; Chang et al. 1992), was found in 3 of 6 G6PD-deficient Chinese from Hawaii. The nt 487 G6PD^{Mahidol}, originally reported from Thailand (Panich et al. 1972; Vulliamy et al. 1989), and in one Chinese from Taiwan (Chang et al. 1992; Tang et al. 1992), was found in two of nine Laotians. The nt 493, reported in Chinese (Tang et al. 1992; Chang et al. 1992), was found in four Filipinos. The nt 871 G6PD^{Jammu} from India (Beutler 1976), or G6PD$^{Viang\ Chan}$ from Laos (Poon et al. 1988) was seen in five Laotians and six Filipinos, who all had the polymorphisms characteristic of G6PD$^{Viang\ Chan}$ (Beutler et al. 1991b, 1992c). The nt 1003 G6PD^{Chatham} (Vulliamy et al. 1988) was seen by us in eight Filipinos. The nt 1376 G6PD$^{Taiwan\text{-}Hakka}$ or G6PD^{Canton}, the most common in Taiwan (Chiu et al. 1991b; Chang et al. 1992; Stevens et al. 1990; Tang et al. 1992), was seen by us in only one Japanese male. The nt 1388 G6PD^{Kaiping}, also common in Taiwan (Chiu et al. 1991a, b; Chang et al. 1992; Tang et al. 1992), was seen in two Chinese and one Laotian.

The nt 1360 mutation (Beutler et al. 1992b) detected in Hawaii was found in 36 of 53 chromosomes from Filipinos, in 2 from Laos, and 1 from Japan; it has also been reported independently in Chinese (Perng et al. 1992), and so must be a common variant, probably a well-characterized biochemical variant (Beutler and Yoshida 1988) that has not yet been identified at the molecular level, such as G6PD^{Union}, or possibly G6PD^{Panay} (Beutler 1990; Fernandez and Fairbanks 1968; Yoshida et al. 1970). Most of those with the nt 1360 mutation had little or no enzyme activity, including the African American subject. Four Oriental males with the nt 1360 mutation had > 1.5 units G6PD activity; two of these had a leucocytosis. The mild nt 835 mutation we found in one Chinese might be a rare variant. The two African Americans with the G6PD A$^-$ variant had 1.5 and 1.9 units activity.

The residual enzyme activity we found in the different mutant types failed to correlate fully with previously reported activity levels (Table 5; Beutler et al. 1988). This could be attributed mostly to minor variations or small sample size, but not for the 12 samples with the nt 871 mutation, where we consistently found much higher activity than reported, equivalent to class 3 rather than class 2 in severity.

Clinical history

None of the 188 males or 37 females with deficient G6PD activity detected in this study had a positive clinical history of neonatal hyperbilirubinemia, deafness or brain damage from kernicterus, episodes of hemolytic anemia, favism, or any other adverse effects on their general health. In Hawaii, prior to this study, a boy of partial Chinese ancestry and a Filipino boy with brain damage from kernicterus due to G6PD deficiency had been seen, one 3-year-old boy had developed acute hemoglobinuria after

aspirin treatment for a fever, and one 8-year-old Filipino boy had collapsed with acute hemolysis after eating about 20 dried fava beans.

Several G6PD-deficient males were also heterozygous for an α- or a β-thalassemia; one Laotian boy with G6PD deficiency had transfusion-dependent Hb H/Constant Spring disease. The presence of heterozygosity or compound heterozygosity for a thalassemia did not seem to affect either the G6PD activity or the expression of any α or β globin genotype.

Conclusions

G6PD deficiency can cause susceptibility to neonatal jaundice and to later fever- or drug-induced hemolytic anemia. Although most patients with G6PD deficiency detected in this screening project had benign histories, G6PD deficiency clearly can have serious consequences. Standard qualitative and quantitative screening tests will detect all samples with deficient activity, and most with low activity. Population screening will find a very large number of G6PD-deficient males and some G6PD-deficient females in Chinese, Filipinos, and Laotians. A variety of molecular mutations account for the deficiencies in each ethnic group. Successful mutation analysis will allow for accurate heterozygote detection and fetal diagnosis of the more serious G6PD deficiencies (Beutler et al. 1992a).

Acknowledgements. We are grateful to the Clinical Laboratories that kindly collaborated in sample collections, and to the physicians who referred patients and their families to us. This work was supported by U.S. Public Health Service MCH Grants MCJ-153562 and MCJ-151002, by NIH grant HL25552, by grants from Kapiolani Medical Center for Women and Children, and from the Sam and Rose Stein Charitable Trust Fund.

References

Beutler E (1966) A series of new screening procedures for pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency, and glutathione reductase deficiency. Blood 28: 553–562

Beutler E (1976) Glucose-6-phosphate dehydrogenase deficiency: a new Indian variant, G-6-PD Jammu. In: Sen NN, Basu AK (eds) Trends in haematology. Bagchi, Calcutta, pp 279–283

Beutler E (1990) The genetics of glucose-6-phosphate dehydrogenase deficiency. Semin Hematol 27:137–164

Beutler E (1991) Glucose-6-phosphate dehydrogenase (G6PD) deficiency. N Engl J Med 324:169–174

Beutler E (1992) Molecular biology of G6PD variants and other red cell enzyme defects. Annu Rev Med 43:47–59

Beutler E, Yoshida A (1988) Genetic variation of glucose-6-phosphate dehydrogenase: a catalog and future prospects. Medicine 67:311–334

Beutler E, Yeh M, Fairbanks VF (1962) The normal human female as a mosaic of X-chromosome activity: studies using the gene for G-6-PD deficiency as a marker. Proc Natl Acad Sci USA 48: 9–16

Beutler E, Kuhl W, Gelbart T, Forman L (1991a) DNA sequence abnormalities of human glucose-6-phosphate dehydrogenase variants. J Biol Chem 266:4145–4150

Beutler E, Prchal JT, Westwood B, Kuhl W (1991b) Definition of the mutations of G6PD Wayne, G6PD Viangchan, G6PD Jammu and G6PD "LeJeune." Acta Haematol (Basel) 86: 179–182

Beutler E, Kuhl W, Fox M, Tabsh K, Crandall BF (1992a) Prenatal diagnosis of glucose-6-P dehydrogenase (G6PD) deficiency. Acta Haematol (Basel) 87:103–104

Beutler E, Westwood B, Kuhl W, Hsia YE (1992b) G6PD variants in Hawaii. Hum Hered 42:327–329

Beutler E, Westwood B, Sipe B (1992c) A new polymorphic site in the G6PD gene. Hum Genet 89:485–486

Chang J-G, Chiou S-S, Perng L-I, Chen T-C, Liu T-C, Lee L-S, Chen P-H, Tang TK (1992) Molecular characterization of glucose-6-phosphate dehydrogenase (G6PD) deficiency by natural and amplification created restriction sites: five mutations account for most G6PD deficiency cases in Taiwan. Blood 80: 1079–1082

Chiu DTY, Zuo L, Chen E, Chao L, Louie E, Lubin B, Liu TZ, Du C-S (1991a) Two commonly occurring nucleotide base substitutions in Chinese G6PD variants. Biochem Biophys Res Commun 180:988–993

Chiu DTY, Zuo L, Chen E, Chao LT, Louie E, Lubin BH, Du CS (1991b) DNA sequence abnormalities in Chinese glucose-6-phosphate dehydrogenase (G6PD) variants (abstract). Blood 78:252

Du CS, Xu YK, Hua XY, Wu QL, Liu LB (1988) Glucose-6-phosphate dehydrogenase variants and their frequency in Guangdong, China. Hum Genet 80:385–388

Fernandez M, Fairbanks VF (1968) Glucose-6-phosphate dehydrogenase deficiency in the Philippines: report of a new variant – G6PD Panay. Mayo Clin Proc 43:645–660

Hirono A, Beutler E (1988) Molecular cloning and nucleotide sequence of cDNA for human glucose-6-phosphate dehydrogenase variant A(–). Proc Natl Acad Sci USA 85:3951–3954

Hsia YE (1991) Detection and prevention of important α-thalassemia variants. Semin Perinatol 15 [Suppl 1]:35–42

Hsia YE, Yuen J, Hunt JA, Rattabasamay P, Hall J, Takaesu N, Titus EAB, Fujita J, Ford C-A (1988) The different types of alpha thalassemia: practical and genetic aspects. Hemoglobin 12:465–484

Hsia YE, Miyakawa F, Baltazar J, Ching NSP, Yuen J, Westwood B, Beutler E (1992) Frequency of G6PD mutations in Chinese, Filipinos, and Laotians (abstract). Am J Hum Genet 50:151

Missiou-Tsagaraki S (1991) Screening for glucose-6-phosphate dehydrogenase deficiency as a preventive measure: prevalence among 1,286,000 Greek newborn infants. J Pediatr 119:293–299

Morelli A, Benatti U, Lenzerini L, Sparatore B, Salamino F, Melloni E, Michetti M, Pontremol S, De Flora A (1981) The interference of leukocytes and platelets with the measurement of glucose-6-phosphate dehydrogenase activity of erythrocytes with low activity variants of the enzyme. Blood 58:642–644

Motulsky AG, Stransky E, Fraser GR (1964) Glucose-6-phosphate dehydrogenase (G6PD) deficiency, thalassaemia, and abnormal haemoglobins in the Philippines. J Med Genet 1:102–106

Nitowsky HM, Soderman DD, Herz F (1965) Glucose-6-phosphate-dehydrogenase deficiency in Filipinos. Lancet I:917

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformational polymorphisms. Proc Natl Acad Sci USA 86:2766–2770

Panich V (1986) G6PD variants in southern Asian populations. In: Yoshida A, Beutler E (ed) Glucose-6-phosphate dehydrogenase. Academic Press, Orlando, Fla, pp 195–241

Panich V, Sungnate T, Wasi P, Na-Nakorn S (1972) G-6-PD Mahidol: the most common glucose-6-phosphate dehydrogenase variant in Thailand. J Med Assoc Thai 55:576–585

Perng L-I, Chiou S-S, Liu T-C, Chang J-G (1992) A novel C to T substitution at nucleotide 1360 of cDNA which abolishes a natural *Hha*I site accounts for a new G6PD deficiency gene in Chinese. Hum Mol Genet 1:205

Persico MG, Viglietto G, Martino G, Toniolo D, Paonessa G, Moscatelli C, Dono R, Vulliamy T, Luzzatto L, D'Urso M (1986) Isolation of human glucose-6-phosphate dehydrogenase (G6PD) cDNA clones: primary structure of the protein and un-

usual 5′ non-coding region. Nucleic Acids Res 14:2511–2522, 7822

Poon M-C, Hall K, Scott CW, Prchal JT (1988) G6PD Viangchan: a new glucose-6-phosphate dehydrogenase variant from Laos. Hum Genet 78:98–99

Sicard D, Kaplan JC, Labie D (1978) Haemoglobinopathies and G-6-PD deficiency in Laos. Lancet II:571–572

Stevens DJ, Wanachiwanawin W, Mason PJ, Vulliamy TJ, Luzzatto L (1990) G6PD Canton, a common deficient variant in Southeast Asia caused by a 459 Arg to Leu mutation. Nucleic Acids Res 18:7190

Tang TK, Huang C-S, Huang M-J, Tam K-B, Yeh C-H, Tang C-JC (1992) Diverse point mutations result in glucose-6-phosphate dehydrogenase (G6PD) polymorphism in Taiwan. Blood 79: 2135–2140

Vulliamy TJ, D'Urso M, Battistuzzi G, Estrada M, Foulkes NS, Martini G, Calabro V, Poggi V, Giordano R, Town M, Luzzato L, Persico MG (1988) Diverse point mutations in the human glucose-6-phosphate dehydrogenase gene cause enzyme deficiency and mild or severe hemolytic anemia. Proc Natl Acad Sci USA 85:5171–5175

Vulliamy TJ, Wanachiwanawin W, Mason PJ, Luzzatto L (1989) G6PD Mahidol, a common deficient variant in Southeast Asia is caused by a (163) glycine→serine mutation. Nucleic Acids Res 17:5868

Yoshida A, Baur EW, Motulsky AG (1970) A Philipino glucose-6-phosphate dehydrogenase variant (G6PD Union) with enzyme deficiency and altered substrate specificity. Blood 35:506–513

Yoshida A, Beutler E, Motulsky AG (1971) Table of glucose-6-phosphate dehydrogenase variants. Bull World Health Organ 45:243–253