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Multiple Glucose 6-Phosphate Dehydrogenase–Deficient Variants Correlate with Malaria Endemicity in the Vanuatu Archipelago (Southwestern Pacific)

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Summary

In studying the relationship between genetic abnormalities of red blood cells and malaria endemicity in the Vanuatu archipelago in the southwestern Pacific, we have found that of 1,442 males tested, 98 (6.8%) were G6PD deficient. The prevalence of GdPD deficiency varied widely (0%–39%), both from one island to another and in different parts of the same island, and generally correlated positively with the degree of malaria transmission. The properties of G6PD from GdPD-deficient subjects were analyzed in a subset of 53 samples. In all cases the residual red-blood-cell activity was <10%. There were three phenotypic patterns. PCR amplification and sequencing of the entire coding region of the G6PD gene showed that the first of these patterns corresponded to G6PD Union (nucleotide 1360C→T; amino acid 454Arg→Cys), previously encountered elsewhere. Analysis of samples exhibiting the second pattern revealed two new mutants: G6PD Vanua Lava (nucleotide 383T→C; amino acid 128Leu→Pro) and G6PD Namoru (nucleotide 208T→C; amino acid 70Tyr→His); in three samples, the underlying mutation has not yet been identified. Analysis of the sample exhibiting the third pattern revealed another new mutant: G6PD Naone (nucleotide 497G→A; amino acid 166Arg→His). Of the four mutations, G6PD Union and G6PD Vanua Lava have a polymorphic frequency in more than one island; and G6PD Vanua Lava has also been detected in a sample from Papua New Guinea. G6PD deficiency is of clinical importance in Vanuatu because it is a cause of neonatal jaundice and is responsible for numerous episodes of drug-induced acute hemolytic anemia.

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Introduction

Glucose 6-phosphate dehydrogenase (G6PD) is a cytosolic enzyme present in all cells. It catalyzes the first step in the hexose monophosphate pathway and, through this reaction, generates NADPH, which is required as an electron donor in various biosynthetic pathways and for the regeneration of reduced glutathione, which helps protect cells against oxidative damage. The production of NADPH by G6PD is of particular importance in red blood cells, which are highly susceptible to oxidative damage where other NADPH-producing enzymes are lacking.

Deficiency of G6PD activity is the most common known enzymopathy, and it is estimated to affect 400 million people worldwide, mainly in tropical and subtropical regions (Vulliamy et al. 1992). More than 300 G6PD variants have been described (Luzzatto and Battistuzzi 1985; Luzzatto and Mehta 1989), many of which reach polymorphic frequencies in affected populations. The high prevalence of independently arisen G6PD mutants is thought to be a result of selection by malaria (Luzzatto 1979; Greene 1993). The gene encoding G6PD maps to the X chromosome, and, therefore, common clinical manifestations such as neonatal jaundice and acute hemolytic anemia are usually more severe in males (Luzzatto and Mehta 1989). G6PD-associated neonatal jaundice is occasionally severe enough to cause death or permanent neurological damage (Luzzatto 1993). Acute hemolytic anemia is precipitated by a number of drugs (e.g., primaquine), infection, or the ingestion of fava beans. However, the majority of individuals with G6PD deficiency are clinically asymptomatic and have never had a hemolytic episode (Beutler 1978).

A previous survey carried out on a group of 100 people from the Emae island in the south of Vanuatu did not detect any G6PD deficient subjects (Bowden et al. 1985). Subsequently, primaquine was introduced into the standard treatment of malaria, for its antigametocytic effect. In the course of a study on the relationship between malaria, thalassemia, and other genetic red-blood-cell abnormalities, cord bloods were screened from two islands in the north of Vanuatu (M. Ganczakowski, unpublished data), and many G6PD-deficient subjects were detected. Cases

of acute hemolysis—previously diagnosed as blackwater fever—were observed in male subjects and were found to occur, not surprisingly, in subjects who were G6PD deficient (Reeve et al. 1992). Therefore, it became a matter of public-health importance to investigate the extent and geographic distribution of G6PD deficiency in Vanuatu.

On grounds of historical and anthropological evidence (Bellwood 1989), the ancestors of the people inhabiting Vanuatu today are thought to have migrated from Papua New Guinea and the Solomon Islands some 4,000–5,000 years ago; and close genetic affinities between Melanesians in Papua New Guinea and in Vanuatu has been substantiated recently, with the advent of DNA analysis (Hill et al. 1985, 1989; Serjeantson and Hill 1989). A total of 22 biochemical variants of G6PD have been reported from Papua New Guinea (Yenchinsomanus et al. 1986). Therefore, the finding of G6PD deficiency in Vanuatu was not surprising, and it became of interest to map it further and to identify the mutations in these islands.

Subjects and Methods

The Republic of Vanuatu is an archipelago of islands forming part of Island Melanesia in the southwestern Pacific. As in most of Melanesia, malaria is endemic, and two islands in the northern part of the archipelago were selected for a large infant cohort study to investigate the effect of genetic red-blood-cell disorders on malaria. As part of this study, G6PD screening was performed on cord bloods from consecutive births at two centers: the Northern District Hospital, on the island of Espiritu Santo, and the Kerepei Health Centre, on the island of Maewo. Of the 705 male babies tested, 31 were found to be G6PD deficient. In addition, blood samples collected from 737 unrelated males from different islands or villages throughout Vanuatu were screened, and 67 were found to be G6PD deficient. Thus, 98 G6PD-deficient males were detected among 1,442 unselected males tested. A further 11 G6PD-deficient males were detected because they presented with clinically significant hemolysis, and 31 more G6PD-deficient males were detected, on screening of family members of the above groups. All samples were tested for G6PD deficiency, in the local laboratory in Espiritu Santo, using the Sigma 400 dye decolorization test. Further biochemical characterization was performed on 53 of the G6PD-deficient samples, either in the laboratory in Vanuatu or, within 4 d, on samples transported on ice to London.

Biochemical Characterization

The following biochemical characteristics were studied, using methods previously described: G6PD activity in erythrocytes, electrophoretic mobility in Tris-borate-EDTA buffer (pH 8.6), 2-deoxy glucose-6-phosphate (2d-G6P) percentage rate, and deamino-nicotinamide adenine dinucleotide phosphate (dNADP) percentage rate (Bekte et al. 1967; Rattazzi et al. 1967; Town 1992).

DNA Analysis

DNA was prepared from peripheral blood, using urea lysis and phenol/chloroform extraction (Sykes 1983). The G6PD-coding regions were amplified from genomic DNA by PCR, using primers as described elsewhere (Poggi et al. 1990; Town 1992), and products of the PCR reactions were examined for SSCPs (Orita et al. 1989; Corcoran et al. 1992). Sequencing was carried out using single-stranded M13 phage clones with the chain termination method (Sanger et al. 1980), using a sequenase kit. G6PD-variant exons were sequenced in both directions and were compared with that of the normal G6PD sequence.

Alteration of a restriction-enzyme site by a mutation was used to screen other DNA samples from G6PD-deficient subjects, for the presence of the mutation. A portion of the reaction product was digested with the appropriate restriction enzyme, according to the manufacturers' instructions, and was then run on 1%–3% agarose gels stained with ethidium bromide and was photographed under UV light. When it was more appropriate, primers were designed for detection of the point mutation by allele-specific priming as follows: for detection of the G6PD Vanua Lava mutation, primers 5′ CAACAGCCACATGGATGACCC and 3′ AGGCGGGAAGGGAGGGC were used; for detection of the G6PD Naone mutation, primers 5′ CAGCAGAGGCTGGAACCA and 3′ CACTACCTGGGCAAGGAG were used. Amplification was performed in Cetus buffer with a final $MgCl_2$ concentration of 1.5 mM, with 30 cycles at 94°C for 45 s, 55°C for 1 min, and 72°C for 1 min.

Some samples (identified as having the G6PD-Union mutation) were haplotyped, using two polymorphic sites lying within the G6PD gene, namely, the silent C→T transition at nucleotide position 1311 and the polymorphic *Nla*III restriction-enzyme site within intron 11. (Filosa et al. 1993).

Results

Prevalence and Distribution of G6PD Deficiency

Of the 1,442 unselected males tested, 98 were found to be G6PD deficient, giving an overall frequency of 6.8%. Marked regional differences were observed (range 0%–39%; see table 1), with little or no G6PD deficiency in the south of Vanuatu and in the small far-northern islands of the Torres group and with high prevalence in the central and northern islands (see fig. 1).

Biochemical Characterization

Biochemical characterization was carried out in a subset of 53 of the G6PD-deficient males. This subset included 10 cord bloods, 30 samples from the population surveys, 4

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Table 1

G6PD Deficiency in Vanuatu

Location	No. of Males	No. G6PD Deficient	Gene Frequency
Santo:			
Cord bloods	609	27	.04
Wunpuko village	18	7	.39
Port Olry village	128	17	.13
Others	96	3	.03
Maewo:			
Cord bloods	96	4	.04
Navisor village	28	3	.11
Others	98	15	.15
Banks:			
Vanua Lava	29	5	.17
Others islands	38	3	.08
Torres Islands	24	0	.00
Efate	130	13	.10
Tanna	100	1	.01
Aneityum	48	0	.00
Overall	1,442	98	.068

patients with acute intravascular hemolysis, and 9 samples from relatives of the above groups. These G6PD-deficient samples were characterized by analysis of the following four properties: (*a*) quantitation of the residual activity in red blood cells; (*b*) electrophoretic mobility—activity on the substrate analogues; (*c*) 2d-G6P; and (*d*) dNADP. The distribution of values for these properties among G6PD-deficient males revealed biochemical heterogeneity, leading to an initial classification of the samples into three distinct phenotypic groupings (fig. 2*a–d*). The largest group of samples had both fast electrophoretic mobility and increased activity with both substrate analogues, dNADP and 2d-G6P. A second group of 16 samples had slow electrophoretic mobility and near-normal activity with the substrate analogues. A single sample had fast electrophoretic mobility but low activity with dNADP and 2d-G6P.

Molecular Characterization

The entire coding region of the G6PD gene from three individuals (one from each of the three biochemically distinct groups defined above) was PCR-amplified and sequenced. In each case, a single point mutation was found, predicting an amino acid substitution in the protein. In a sample from the first group, we found a substitution of C→T in exon 11 at nucleotide position 1,360, resulting in an amino acid change of arginine to lysine at position 454. This mutation causes the loss of an *Hha*I restriction-enzyme site, and this property was used to test 36 additional samples with the same biochemical phenotype (fig. 3*a*). The presence of the same mutation was confirmed in all samples. We had originally named this molecular variant *G6PD Maewo* (Calabrò et al. 1993), but it has since become apparent that this mutation corresponds to the variant previously described as *G6PD Union* (Rovira et al. 1994).

The sample from the second biochemical group had a single base substitution in exon 5, T→C at position 383, resulting in an amino acid change of leucine to proline at position 128: we named this new variant *G6PD Vanua Lava*. The mutation results in the loss of an *Mnl*I restriction-enzyme site. Because several *Mnl*I sites occur in exon 5, interpretation of restriction patterns is difficult: therefore, we used allele-specific priming for testing further DNA samples (fig. 3*b*). Of the 16 individuals with a similar biochemical phenotype analyzed in this way, the Vanua Lava mutation was detected in 11, indicating that other mutation(s) must be present in this phenotypic group. By SSCP analysis, we found in one of the samples from this group, which lacked the Vanua Lava mutation, a band

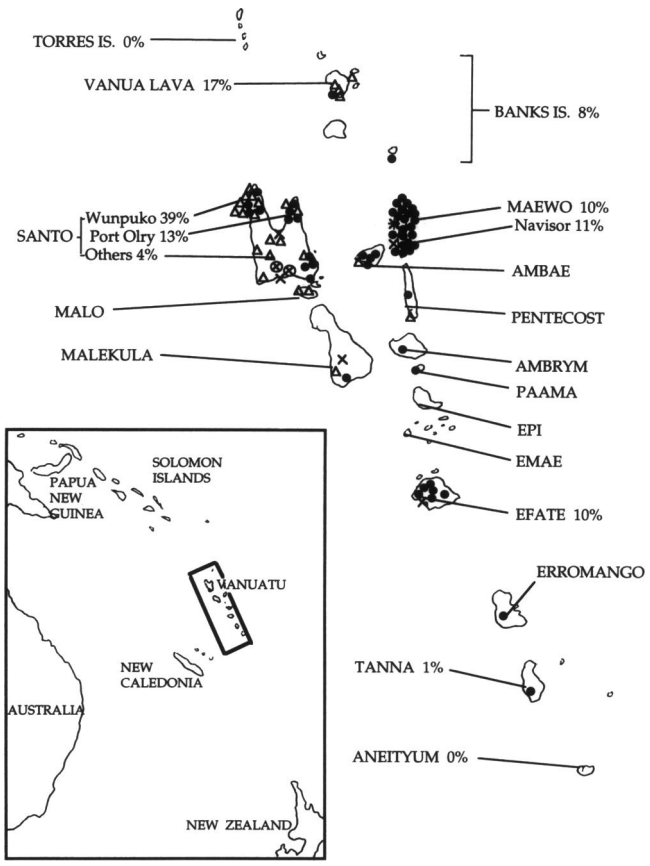

Figure 1 Map of Vanuatu, showing numbers and percentages of G6PD-deficient males at different locations and distribution of the mutations. Triangles (Δ) represent G6PD Vanua Lava; blackened circles (●) represent G6PD Union, circles containing × marks (⊗) represent G6PD Namoru; and asterisks (*) represent G6PD Naone. Each point represents a G6PD deficient male in whom the mutation has been determined at the molecular level. "×" marks locations of G6PD-deficient males in whom the above mutations have been excluded and whose mutations have not yet been characterized.

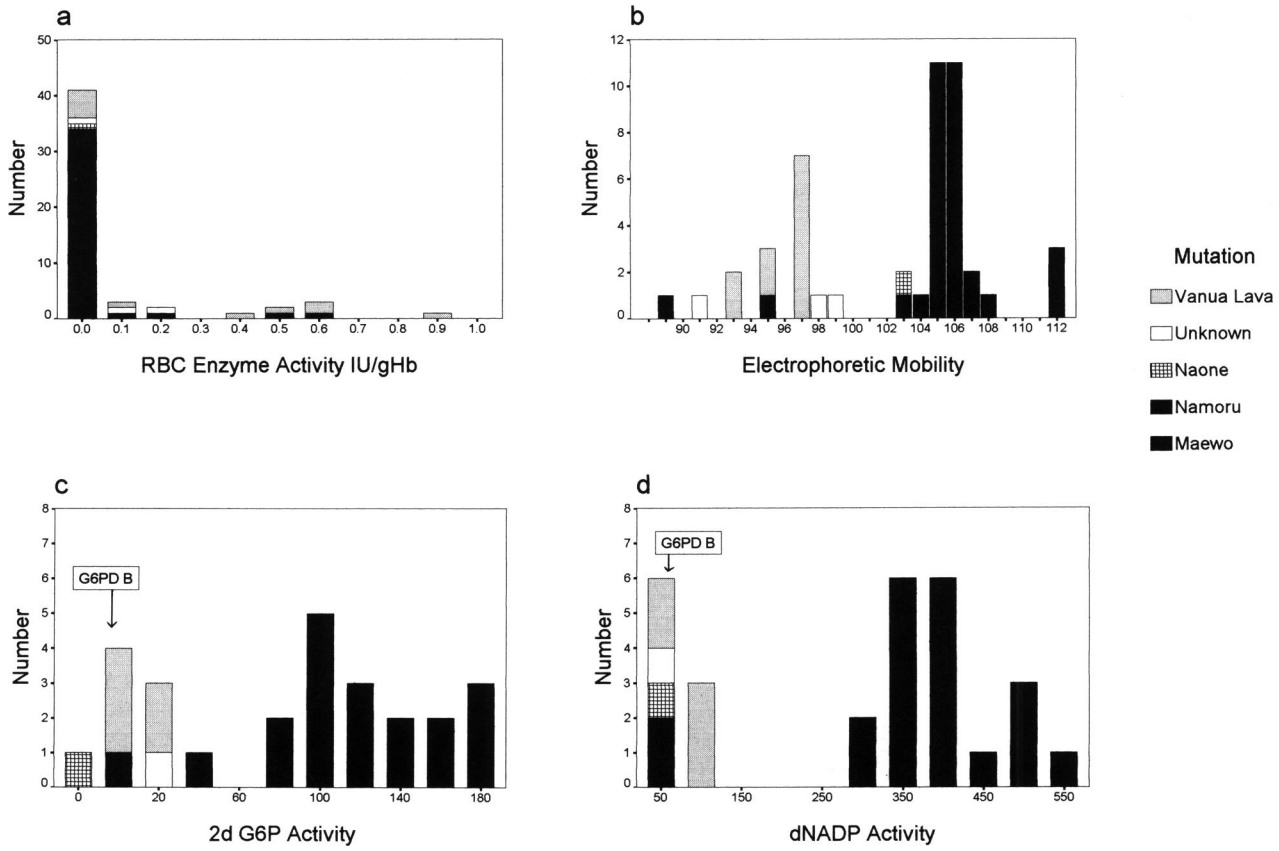

Figure 2 Distribution of biochemical characteristics of G6PD-deficient enzymes in Vanuatu. *a*, Distribution of red-blood-cell enzyme activity in IU/g Hb. *b*, Distribution of electrophoretic mobility (% mobility with G6PD B). *c*, Distribution of activity with 2d-G6P (% activity with G6P). *d*, Distribution of activity with d-NADP (% activity with NADP).

shift in G6PD exon 4. Direct sequencing revealed a mutation of T→C at position 208 in this exon, resulting in the substitution of histidine for tyrosine at amino acid 70: we have called this new variant *G6PD Namoru*. A *Nla*III restriction-enzyme site is created by this mutation (fig. 3*c*), and, by using this enzyme, we found the Namoru mutation in one other sample. This leaves three samples from this phenotypic group, which are negative for both the G6PD Vanua Lava and the G6PD Namoru mutations, indicating that there is at least one other, yet unidentified, mutation that results in this phenotype.

The single individual with the third biochemical phenotype had a single-base-pair substitution in exon 6, G→A at position 497, resulting in the substitution of histidine for arginine at amino acid position 166. We named this new variant *G6PD Naone*. By allele-specific priming (fig. 3*d*), this mutation was detected in other members of this subjects family. The characteristics of the four mutants are shown in table 2.

G6PD Union Haplotype

Two polymorphic sites lying within the G6PD gene were sought in 21 of the samples from males with the G6PD Union mutation. The results are shown in table 3. Five G6PD Union samples from Maewo Island all had the same haplotype: 1311^- and *Nla*III^+. The five samples from Efate Island differed in that they were all negative for the *Nla*III polymorphic site. The samples from Maewo were geographically spread throughout this small island, whereas the samples from Efate were all from the same village of Erakor. The other 11 samples, mainly from the island of Espiritu Santo, showed a mixture of the two haplotypes.

Clinical Significance

Over a period of 18 mo, in the islands of Espiritu Santo and Maewo, acute hemolysis occurred after administration of a single dose (45 mg) of primaquine in 11 G6PD-deficient male adults; and further cases were observed by health workers in other parts of Vanuatu (Reeve et al. 1992). DNA from the 11 cases in this study has been tested for the presence of each of the four known G6PD mutations. Five of the patients had G6PD Union, three had G6PD Vanua Lava, and in the remaining three, none of the four mutations described above was present.

Babies recruited into the infant cohort study were ex-

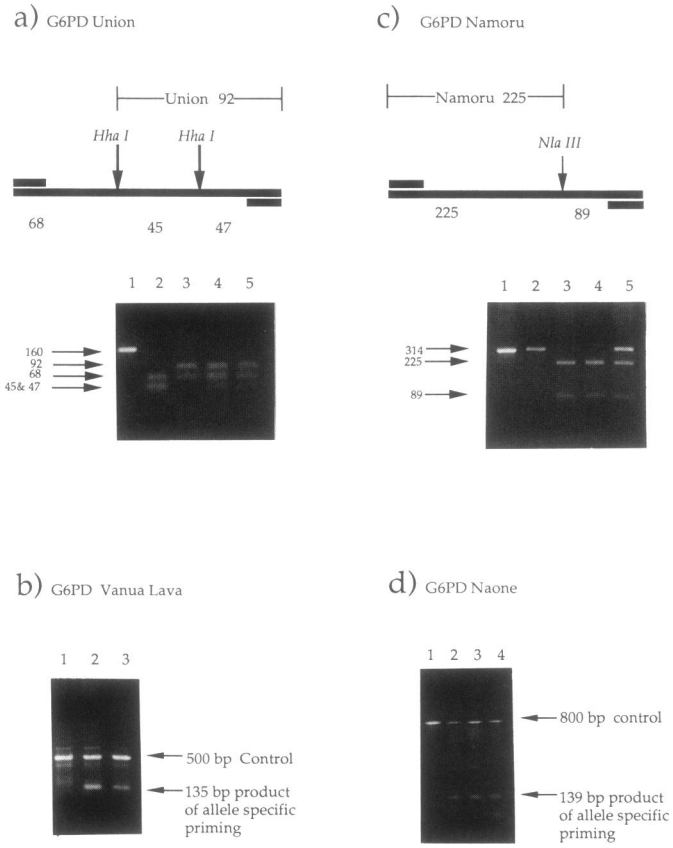

Figure 3 Screening for the G6PD mutations in further DNA samples. *a,* Diagrammatic illustration of the PCR amplification product of exon 11, indicating *Hha*I enzyme sites and the size of restriction fragments. In G6PD Union a *Hha*I site is destroyed, as indicated by the presence of a band of new fragment of 92 bp. Agrarose gel electrophoresis shows the following: undigested PCR product (lane 1), normal results (lane 2), Union mutation in a G6PD-deficient male (lanes 3), and heterozygous females (lanes 4 and 5). *b,* Agarose gel electrophoresis of products of allele-specific priming for the Vanua Lava mutation. A band at 135 bp indicates the presence of the Vanua Lava mutation. Lane 1 shows normal results, while lanes 2 and 3 show the Vanua Lava mutation. Control primers that give a 500-bp product were used to confirm DNA amplification of all samples. *c,* Diagrammatic illustration of the PCR amplification product of exons 3 and 4. A *Nla*III site is created by the G6PD Namoru mutation, giving two restriction fragments after digestion of 225 bp and 89 bp. G6PD B yields a single product of 314 bp. Agarose gel electrophoresis after *Nla*III digestion shows the following: undigested PCR product (lane 1), normal results (lane 2), Namoru mutation in a G6PD-deficient male (lane 4), and a heterozygous female (lane 5). *d,* Agarose gel electrophoresis of products of allele-specific priming for the Naone mutation. A band at 139 bp indicates the presence of the Naone mutation. Lane 1 shows normal results, while lanes 2 and 3 show the Naone mutation. Control primers giving a PCR product of 800 bp were used to confirm DNA amplification of all samples.

amined for ≤2 days after birth. Of 19 G6PD-deficient babies, 4 were clinically jaundiced. Thus, the incidence of neonatal jaundice (NNJ) among G6PD-deficient babies was 21%, and this is likely to be an underestimate, as the babies went home within 1 or 2 d of birth and were not examined at day 3, when NNJ associated with G6PD deficiency reaches a peak (Meloni et al. 1975). DNA analysis of the G6PD-deficient babies with NNJ revealed G6PD Union in three and G6PD Vanua Lava in one.

Discussion

This study confirms that G6PD deficiency is prevalent in Vanuatu and that it is responsible for acute hemolytic anemia following primaquine administration. As a result of these findings, primaquine has been withdrawn as part of the standard treatment of malaria. In addition, the finding that G6PD deficiency is strongly associated with NNJ in this region, as elsewhere (Matthay and Mentzer 1981), highlights the public health importance of this genetic abnormality.

Heterogeneity of G6PD Deficiency

Biochemical analysis has revealed heterogeneity of G6PD deficiency in Vanuatu, with at least three distinct biochemical phenotypes. Subsequent DNA analysis has fully validated the homogeneity of one of the phenotypic groups, because all subjects in this group had the G6PD Union mutation. By contrast, in the second phenotypic group, we have found considerable molecular heterogeneity, including two new mutants, G6PD Vanua Lava and G6PD Namoru, and at least one other, as yet unidentified, mutant. A single subject formed the third phenotypic group, and he had a different mutant, G6PD Naone, which was also traced through his family.

G6PD Union is of special interest because, having been originally described in an individual from the Philippines (Yoshida et al. 1970), it has subsequently been found not only in Vanuatu but also in southern Italy (Calabrò et al. 1993), in China (Perng et al. 1992), in Hawaii (Hsia et al. 1993), and in Gypsies in Spain (Rovira et al. 1994). Thus, it has emerged as the only G6PD-deficient variant thus far with a worldwide geographic distribution. While it is possible, in principle, that it has a single origin, for this to be true it would have to be very ancient indeed in the history of mankind. It seems perhaps more likely that the G6PD Union mutation has arisen independently more than once, particularly as the mutation is in a GC dinucleotide, which may have a greater than average mutation rate as a result of methyl cytosine deamination (Schorderet and Gartler 1992). The fact that the G6PD Union mutation is found on two different haplotypes in Vanuatu can be regarded as evidence favoring independent mutations. By contrast, the other three variants reported here are all new, and they are thus far characteristic of the Vanuatu people, although they may not necessarily have originated there. Indeed, in view of the fact that Melanesians may have migrated to Vanuatu from Southeast Asia via Papua New Guinea (Serjeantson and Hill 1989), it is interesting that we have found G6PD Vanua Lava in a sample from the north coast of

Table 2

Characteristics of G6PD-Deficient Variants in Vanuatu

Variant	Nucleotide Change	Exon	Amino Acid Change	Restriction-Enzyme Site	Change in Net Charge	RBC Activity: IU/g Hb	RBC Activity: % Normal G6PD[a]	Electrophoretic Mobility[a] (% Normal G6PD)	dNAPD Activity[b]	2d-G6P Activity[b]
Union	1360 C→T	11	454 Arg→Cys	*Hha*I destroyed	−1	<.1 ± .1	<1% (36)	106 ± 2 (31)	400 ± 64 (19)	127 ± 34 (17)
Vanua Lava	383 T→C	5	128 Leu → Pro	*Mnl*I destroyed	No change	.36 ± .3	4% (11)	96 ± 2 (11)	63 ± 22 (5)	18 ± 3 (5)
Naone	497 G→A	6	166 Arg→His	*SfaN*I destroyed	−1[c]	.12	1% (1)	103 (1)	49 (1)	2 (1)
Namouru	208 T → C	4	70 Try → His	*Nla*III created	+1	.36 ± .3	4% (2)	92 ± 3 (2)	48 ± 7 (2)	30 ± 20 (2)
Mutation Not Any of the Above (Yet to be Characterized):										
Subject 1						.11	1% (1)	99 (1)	31 (1)	26 (1)
Subject 2						.0	<1% (1)	98 (1)		
Subject 3						.2	2% (1)	91 (1)		
Control						9.3 ± .3	100%	100	54–60	7–8

[a] Numbers of samples analyzed in parentheses.
[b] Expressed as a percent of activity with normal substrate NADP/G6P.
[c] At physiological pH.

Papua New Guinea. In addition, G6PD Weewak and G6PD Kar Kar (Chockkalingam et al. 1982) are phenotypically similar to G6PD Vanua Lava, although, unfortunately, we have been unable to obtain samples of these variants to investigate whether they have the same mutation. We also note that the Markham variant from Papua New Guinea (Chockkalingam and Board 1980) is phenotypically similar to G6PD Union, and the corresponding mutation has been found in samples from Papua New Guinea (G. Wagner and P. Board, personal communication). Thus, the analysis of G6PD variants is generally in keeping with the presumed origin of the population of Vanuatu.

Table 3

DNA Polymorphic Sites in Males with the G6PD Union Mutation

Sample	Island of Origin	1311	*Nla*III
1	Maewo	−	+
2	Maewo	−	+
3	Maewo	−	+
4	Maewo	−	+
5	Maewo	−	+
6	Efate	−	−
7	Efate	−	−
8	Efate	−	−
9	Efate	−	−
10	Efate	−	−
11	Espiritu Santo	−	+
12	Espiritu Santo	−	−
13	Espiritu Santo	−	−
14	Espiritu Santo	−	−
15	Espiritu Santo	−	−
16	Espiritu Santo	−	+
17	Espiritu Santo	−	−
18	Espiritu Santo	−	+
19	Espiritu Santo	−	−
20	Ambrym	−	+
21	Ambae	−	−

The three new mutations reported here are to be added to the database of ~60 G6PD variants so far identified at the molecular level. Because the three-dimensional structure of the enzyme is not yet known, our ability to interpret why these mutations cause G6PD deficiency can be based on empirical considerations only. G6PD Vanua Lava introduces a proline residue in a region that is probably an α-helix (Persico et al. 1986); therefore, it may cause instability of the protein. G6PD Namoru (amino acid 70) is very near the mutation at amino acid 68, which, together with the mutation of G6PD A, causes it to become deficient in G6PD A−. The mutation of G6PD Naone (amino acid 166) is very near those of three other variants associated with G6PD deficiency: namely, G6PD Mahidol, G6PD Plymouth (amino acid 163), and G6PD' Chinese-3' (amino acid 165). Thus, it is possible that this region of the molecule is critical for its stability.

Epidemiology of G6PD Deficiency and of Malaria

The overall frequency of G6PD deficiency is remarkably similar in Papua New Guinea (6.7% in males) and Vanuatu (6.8% in males). In both areas, there is considerable regional variation in the prevalence of G6PD deficiency (Yenchinsomanus et al. 1986), and in both areas this roughly mirrors malarial endemicity. Malaria is more common, with year-round transmission and an infection rate in young children of nearly one episode per child per year (M. Ganczakowski, unpublished observations) in the

Table 4

Numbers and Percentages of G6PD-Deficient Genes for Which DNA Samples Have Been Screened for the Four Known Mutations

G6PD Mutation	No. of G6PD-Deficient Males	No. of G6PD-Deficient Genes in Females	Total No. of G6PD-Deficient Genes	% of All G6PD-Deficient Genes
Union	50	56	106	60
Vanua Lava	26	30	56	32
Namoru	2	2	4	2
Naone	1	4	5	3
Mutation not characterized	5	1	6	3

larger of the central and northern islands of Vanuatu, and it is on these islands that most of the G6PD deficiency is found. In the southern islands of Tanna and Aneityum, where malaria transmission is more seasonal and where, in some years, little or no transmission occurs, the frequency of G6PD deficiency is very low. G6PD deficiency was also absent from the northernmost islands of Vanuatu, the Torres group, where malaria transmission is also more episodic, presumably because of the low population density (only 500 people spread over four islands). In addition, a previous study in Fiji, which historically has been free of malaria, only 1 G6PD-deficient male (0.11%) was found among 913 indigenous Fijian males of predominantly Melanesian origin (Buchanan et al. 1973).

From the point of view of population genetics, it is clear that the coexistence of different polymorphic variants associated with G6PD deficiency in the Vanuatu archipelago reflects, once again, convergent evolution for a trait advantageous in heterozygotes. G6PD Union and G6PD Vanua Lava are the most common mutant alleles, and together they account for >90% of G6PD deficiency overall (table 4). The geographic distribution of these two mutants is different, with G6PD Union being more common in the eastern islands and G6PD Vanua Lava more common in the western islands (fig. 1). However, the two variants coexist within some islands, and even within villages, where we have observed their segregation in individual families, with the occurrence of compound heterozygotes in women.

Finally, we note that in Vanuatu, just as in other areas (Calabrò et al. 1993; Nafa et al. 1994), limited biochemical characterization has proved its worth as a first step in identifying G6PD mutations in an area previously unexplored in this respect. The levels of residual enzyme activity, electrophoretic mobility, and activity on substrate analogues can be determined with relatively simple equipment in any laboratory. The set of properties emerging from these tests can then be used as a guide in the selection of samples to be subjected to molecular analysis. It is only after the biochemical findings are matched to particular mutations that further testing can be carried out directly on DNA samples, for conclusive identification of a particular variant.

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