

# Characterization of Mutations and Polymorphisms in the G6PD Gene among Filipino Newborns with Glucose-6-Phosphate Dehydrogenase Deficiency

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## ABSTRACT

**Background.** Glucose-6-phosphate (G6PD) deficiency is the most prevalent enzyme deficiency to date. The global prevalence of G6PD deficiency is estimated at around 330 million people affected with the disease worldwide. This 4.9 percent prevalence, correlates highly with geographic areas endemic to malaria. It is the most common among the disorders in the Newborn Screening (NBS) panel in the Philippines, with one confirmed case for every 52 newborns (1:52). This paper determines the molecular background of G6PD deficiency among Filipino newborns detected by newborn screening.

**Methods.** A total of 200 cases confirmed to have G6PD deficiency, 180 males and 20 females, were identified through the Philippine Newborn Screening Program from 2001-2003. Genomic DNA was extracted from dried blood spots followed by multiplex polymerase chain reaction using multiple tandem forward primers and a common reverse primer (MPTP) to detect previously reported common mutations and polymorphisms in exons 5, 6, 9, 11 and 12 of the G6PD gene.

**Results.** Of the 200 samples analyzed, mutations and polymorphisms in the G6PD gene were identified in 148 cases (74%). The most common mutation was a G to A transition on nucleotide 871 (Viangchan) of exon 9 in combination with a silent mutation on exon 11, accounting for 32.9% of the cases. This was followed by a C to T transition on nucleotide 1360 (Union) in 21.1 % of the cases. Other mutations were Vanua Lava in 10%, Chatham in 9.4%

and Canton in 3.5% of the newborns. The silent polymorphism on nucleotide 1311 was present in 12.9% of cases. There were combinations of these mutations and polymorphisms present in a minority of cases.

**Conclusion.** Results of this study showed the molecular heterogeneity underlying G6PD deficiency among Filipino newborns.

**Key Words:** *Glucose-6-phosphate dehydrogenase deficiency, G6PD, mutations, Filipino*

## Introduction

Glucose-6-phosphate (G6PD) deficiency is the most prevalent enzyme deficiency to date. A meta-analysis by Nkhoma et al. in 2009 on the global prevalence of G6PD deficiency estimated around 330 million people affected with the disease worldwide. This 4.9 percent prevalence correlates highly with geographic areas endemic to malaria.<sup>1</sup> It is the most common among the disorders in the Newborn Screening (NBS) panel in the Philippines with one confirmed case for every 52 newborns (1:52) as of December 2010.<sup>2</sup> The enzyme G6PD, which is expressed in all tissues, controls the first step of the pentose phosphate pathway catalyzing the conversion of glucose-6-phosphate to 6-phosphogluconate and at the same time reduces NADP to NADPH.<sup>3</sup> The enzyme is essential in the overall cell metabolism because it maintains an adequate supply of NADPH and protects cells against oxidative stress. Although found in most cells, its function is most important in red blood cells which have no other source of NADPH.<sup>1</sup>

G6PD deficiency is found to be more common in tropical countries where malaria infection is endemic because of selective heterozygote advantage.<sup>1,4,5</sup> It has been hypothesized that G6PD deficiency may have started as a protective factor against malaria. There have been varied results in studies that associate G6PD deficiency with malaria. Some studies have shown a decrease in risk of severe malaria in hemizygous males while showing an inconsistent decrease in risk in heterozygous females. For

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the uncomplicated malaria, studies have shown inconsistent results in the decrease in risk in both hemizygous males and heterozygous females.<sup>4,5</sup> Although most affected individuals are asymptomatic, there is a risk of neonatal jaundice and acute hemolytic anemia triggered by infection, exposure to certain drugs and chemicals, and intake of certain foods like fava beans, soya foods, bitter gourd (ampalaya), menthol, red wine, and tonic water.<sup>2,6</sup>

Diagnosis of G6PD deficiency is based on an enzymatic assay and on the identification of the specific mutation in the G6PD gene. The identification of the genetic variation can help in the management of G6PD patients.<sup>7</sup> This can guide the selection of drugs or treatment protocols that can minimize harmful side effects.

The G6PD gene is mapped to the telomeric region of the long arm of the X-chromosome, Xq28.<sup>8</sup> It has 13 exons and 12 introns spread over a region of 19 kb with an open reading frame of 1545 nucleotides.<sup>9</sup> G6PD deficiency follows an X-linked pattern of inheritance. A remarkable feature of G6PD deficiency is its extreme molecular heterogeneity. Different variants of G6PD deficiency have reached polymorphic frequencies in different parts of the world, secondary to the relative protection they confer against malaria.<sup>10</sup> To date, 193 mutations have been documented.<sup>11</sup> The molecular characterization of G6PD has been reported in many populations;<sup>12-23</sup> however, only few studies involving a small sample population have been reported on G6PD deficiency among Filipinos.<sup>24-27</sup>

Identification of mutations in the G6PD gene is performed as an epidemiologic investigation of G6PD deficiency in many countries. This paper determines the molecular background of G6PD deficiency among Filipino newborns detected by newborn screening.

## Methods

### Subjects

Dried blood spot samples from G6PD deficient newborns were obtained from 180 males and 20 females identified through the Philippine Newborn Screening Program from 2001-2003. These samples were screened for G6PD deficiency using the Formazan ring method.<sup>28</sup> Confirmation of positive cases was done by a red cell based semi-quantitative enzyme assay (Roche).

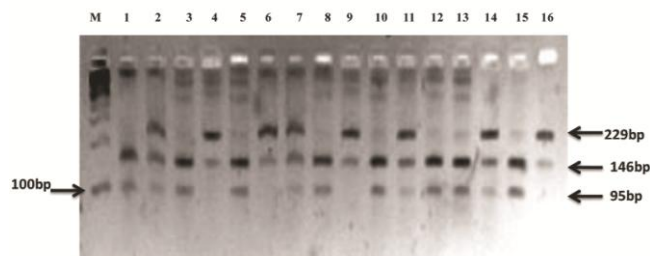
### DNA Mutation Analysis

Genomic DNA was prepared from dried blood spot samples using the DNAzol kit (GIBCO BRL Life Technologist). A normal male Filipino newborn was used as control. Multiplex polymerase chain reaction using multiple tandem forward primers and a common reverse primer (MPTP) was used to detect previously reported common mutations in exons 5, 6, 9, 11 and 12 of the G6PD gene as previously described.<sup>26-28</sup> With MPTP, the absence of the

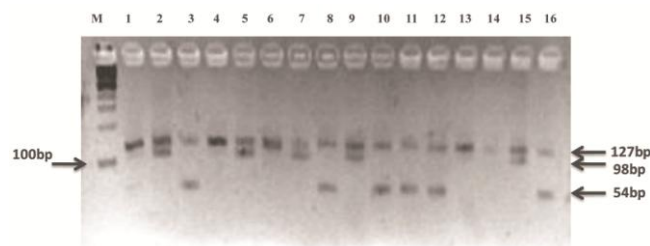
amplified product defines the site of a mutation within a narrow region of the primer recognition site. Amplified products were electrophoresed on a 4% agarose gel containing 0.5 µg/mL of ethidium bromide. Gels were photographed using a UV transilluminator equipped with the Electrophoresis Documentation Analysis System (EDAS).

## Results

Of the 200 Filipino newborns with G6PD deficiency, 148 (74%) had detectable mutations and polymorphisms on exons 5, 6, 9, 11 and 12 of the G6PD gene while the mutation was uncharacterized in 30 samples (15%). The most common mutation, accounting for 32.9% of the cases, was the G to A transition on nucleotide 871 (Viangchan) of exon 9 in combination with a silent mutation in nucleotide 1311 of exon 11 (Figure 1). This was followed by the C to T transition on nucleotide 1360 (Union) in 21.1%. Other mutations included 383 T>C (Vanua Lava) in 10%, 1003 G>A (Chatham) in 9.4 % and 1376 G>T (Canton) in 3.5% of the newborns. The silent polymorphism on nucleotide 1311 was also quite commonly seen in 12.9% of cases (Figure 2). There were combinations of these mutations and silent polymorphisms present in a minority of cases (Table 1).



**Figure 1.** Amplified products of exon 9 of the G6PD gene by MPTP. Lane M is the 100bp marker; lanes 1-13 represent patient samples; lane 14 is the normal control; lane 15 is a known patient with a G>T in the c.871 (G6PD Viangchan); and lane 16 is a known patient with a G>A in c.1003 (G6PD Chatham).



**Figure 2.** Amplified products of exon 11 of the G6PD gene by MPTP. Lane M is the 100bp marker; lanes 1-13 represent patient samples; lane 14 is the normal control; lane 15 is a known patient with a C>T in the c.1311 (G6PD Silent); and lane 16 is a known patient with a C>T in c.1360 (G6PD Union).

## Discussion

G6PD deficiency is one of the most common heritable disorders in humans. Being an X-linked recessive disorder, G6PD deficiency is mostly found in males and accounts for the biased gender distribution of patients. Female patients are either homozygous for the disorder to manifest G6PD deficiency or may have skewed X-inactivation in this X-linked gene. Females who are heterozygous for the disorder show varying degrees of G6PD activity.

The prevalence of G6PD deficiency is highest in areas endemic to malaria because of selective heterozygote advantage. It was previously hypothesized that G6PD deficiency may have started as a protective factor against malaria. It was noted in Indonesia that the incidence of G6PD deficiency was 4.6% in malaria-endemic areas but was only 0.9% in non-endemic areas.<sup>29</sup> This provides a clue that the mutations in the G6PD gene may have been an evolutionary adaptation to a life-threatening infection caused by *Plasmodium falciparum*.

The G6PD gene consists of 13 exons with 1,545 base pairs reading frame.<sup>30</sup> The deficiency is caused mostly by single-base changes at the locus Xq28, which results in an amino-acid change in the G6PD enzyme composed of 515 amino acids. One hundred ninety-three mutations in the G6PD gene have been listed to date.<sup>11</sup>

An individual with G6PD deficiency is prone to a variety of symptoms caused by lysis of the red blood cells after or during an infection, ingestion of certain foods or drugs and upon exposure to certain oxidative substances.

**Table 1.** Distribution of G6PD mutations and polymorphisms among Filipino newborns

G6PD variant	Mutation/Polymorphism	Number	Prevalence (%)
Viangchan/Silent	871 G>A/1311 C/T	56	32.9
Union	1360 C>T	36	21.1
Vanua Lava	383 T>C	17	10
Chatham	1003 G>A	16	9.4
Canton	1376 G>T	6	3.5
Union/Viangchan	1360 C>T/871 G>A	3	1.7
Mediterranean/ Viangchan/Silent	563 C>T/871 G>A/1311 C/T	2	1.1
Mediterranean/Silent	563 C>T/1311 C/T	2	1.1
Union/Chatham	1360 C>T/1003G>A	2	1.1
Mahidol	487 G>A	2	1.1
Vanua Lava/Silent	383 T>C/1311 C/T	1	0.5
Union/Vanua Lava	1360 C>T/383 T>C	1	0.5
Viangchan/ Vanua Lava/Silent	871 G>A/383 T>C/1311 C/T	1	0.5
Viangchan/Canton	871 G>A/1376 G>T	1	0.5
Union/Canton	1360 C>T/1376 G>T	1	0.5
Coimbra/Silent	592 C>T/1311 C/T	1	0.5
Silent	1311 C/T	22	12.9
Uncharacterized		30	15
<b>Total</b>		<b>200</b>	<b>100</b>

We identified 148 mutations (74%) in the G6PD gene among the Filipino newborns included in this study. Fifteen percent (30) of the newborns with G6PD deficiency have yet

uncharacterized mutations. G6PD Viangchan/Silent, Union, Vanua Lava, and Chatham are the most frequent mutations with 32.9%, 21.1%, 12.9%, 10%, and 9.4% prevalence, respectively. The silent polymorphism was also quite commonly seen and was detected in 12.9% of the cases.

The G6PD Viangchan (871 G>A) was first identified in a homogenous ethnic group (Lao) in the province of Viangchan, Laos. Its manifestations include severe enzyme deficiency, normal electrophoretic mobility, increased pH optimum, and abnormal kinetics for the natural substrates G6PD and NADP, as well as the artificial substrates 2-deoxy G6PD and deamino NADP.<sup>31</sup> It is commonly found in Southeast Asian countries and is the most predominant mutation in Laos,<sup>14</sup> Thailand,<sup>15</sup> Cambodia,<sup>19</sup> Malaysia,<sup>17</sup> and Vietnam;<sup>22</sup> and with a lesser degree of prevalence in China<sup>32</sup> and Indonesia.<sup>33</sup> This common 871 G>A variant of the G6PD gene account for 90% of the deficient cases in the mutually exclusive ethnic groups of Laos, Thailand, and Vietnam.

The 1360 C>T mutation on exon 11 of the G6PD gene was first reported in Filipinos living in Hawaii.<sup>25</sup> This mutation corresponded to the variant G6PD Union which was originally found in a Filipino male and was named after the Philippine province of origin of the patient.<sup>24</sup> It has a worldwide distribution and commonly occurs along with other variants.<sup>34</sup> Data from previous studies reveal high incidence rates in the countries in the Pacific Ocean. It was previously reported by Silao et al that the G6PD Union appeared to be the dominant variant in the Philippines.<sup>26</sup> Our study now shows that it is only the second most common mutation. This discrepancy may be reconciled with the fact that our current study has a bigger sample size and may better reflect the true frequency of the G6PD variants.

Polymorphisms have been identified in the human G6PD gene. Seven of such have been reported.<sup>35-38</sup> Among the Filipino newborns included in this study, the SNP identified was 1311 C/T on exon 11, also known as G6PD Silent. Majority of these polymorphisms occurred together with another mutation causing G6PD deficiency, particularly the 871 G>A (Viangchan) on exon 9. These findings were still consistent with previous reports which demonstrated variants of the G6PD gene showing two or more mutations involving different codons;<sup>10,11,16,39</sup> combination with a polymorphism may also be present. However, in 22 of 170 or 12.9%, the silent polymorphism occurred singly and not in combination with a mutation in another exon. A comparison on the effects of a polymorphism in combination with another mutation that cause a selectable phenotype will be more useful and such functional studies should be a part of future studies. Also, this finding may provide an experimental evidence of the possible founder effect of the 871 G>A mutation in our population.

The high prevalence of the Viangchan and Union variants accounting for more than 50% of the cases in the

Philippines are similar to the distribution of these variants in neighboring countries.

The Filipinos may have also inherited the G6PD variants Vanua Lava, Chatham, and Canton from neighboring countries possibly due to intermarriage and migration. A correlation study of prevalence of G6PD variants among minority groups in the Philippines may provide a compelling anthropologic clue into the migration and origin of Filipinos. A correlation study between frequencies of G6PD variants among areas of malarial endemicity may be helpful in determining environmental influence on the mutation of the G6PD gene.

Results of this study demonstrated the molecular heterogeneity underlying G6PD deficiency among Filipino newborns. However, this mutation analysis method which only screened 5 exons for mutations and polymorphisms in the G6PD gene was able to confirm the presence of mutations in 74% male and female G6PD screen positive cases. Further molecular analyses need to be done to find yet undefined mutations in other exons of the G6PD gene.

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