Full Length Research Paper

Common mutations in G6PD of Vietnamese-Kinh deficient patients

Nguyen Thi Hue¹*, Dang Thi Lan Anh¹, Huynh Le Thao Trinh¹ and Phan Ngo Hoang²

¹School of Biotechnology, International University, Vietnam National University, Ho Chi Minh City, Vietnam. ²University of Science, Vietnam National University, Ho Chi Minh City, Vietnam.

Accepted 13 March, 2013

This study was conducted to identify the common mutations occurring within the G6PD gene in the G6PD - Vietnamese deficient patients, which may be the main causative mutations of the G6PD deficiency in Vietnamese-kinh. Sequencing was performed to detect mutations in the coding region of G6PD gene for 30 Vietnamese-Kinh G6PD deficient patients. Seven sets of primers were designed to amplify and sequenced 13 coding regions of G6PD gene. Seven known types of mutations were detected. Each patient had at least one mutation in his G6PD gene. The most common mutation is the silent mutation 1311C>T with frequency of 56.66%. With the high frequency, the 1311C>T mutations are suggested as potential markers for G6PD deficiency in Vietnamese-kinh population.

Key words: G6PD deficiency, Vietnamese-Kinh, 1311C>T, Viangchan, Canton.

INTRODUCTION

G6PD deficiency is considered as the most common hereditary erythroenzymopathy in human with about 500 million individuals affected worldwide (Minucci et al., 2007; Sirdah et al., 2012b). A current global prevalence for G6PD deficiency revealed in a recent systematic review is about 4.9% (Nkhoma et al., 2009). This enzyme deficiency includes many manifestations and the main clinical one is hemolytic anemia accompanied with neonatal jaundice (Noori-Daloii et al., 2006). Depending on the enzyme’ activity level, the World Health Organization (WHO) classified this deficiency into four categories. Class I deficiency usually occurs with chronic non-spherocytic hemolytic anemia (CNSHA) and is quite rare. In contrast, classes II and III are the most prevalent defects with the acute hemolytic anemia (the phenomenon in which anemia is revealed when the body is in an oxidative stress), and class IV is usually asymptomatic with mild deficient or increased enzyme’ activity.

G6PD is an important enzyme in the pentose phosphate pathway (PPP) that supplies reducing energy to cells by reproducing NADPH and indirectly contributing to maintain the intracellular redox potential that helps the cells to resist against the oxidative pressure. Especially important with erythrocytes, the cells are at high risk of oxidative stress due to the oxygen carrying responsibility. These suggest that complete absence of the G6PD enzyme is incompatible with life (Luzzatto and Poggi, 2008). According to previous studies, G6PD deficiency is caused by mutations occurring within G6PD gene that lead to the changes in the enzyme structure. Today, 186 mutations within G6PD gene have been detected (Minucci et al., 2012). All G6PD deficiency causing mutations disturb the coding regions and most of these mutations are single nucleotide polymorphisms (SNPs) that can create amino acid substitutions. The large deletions and frame-shift mutations leading to the complete lacking of the G6PD functionality have not been observed yet. Moreover, there is no mutation occurring in the active sites or the promoter sites and the de novo mutations (causing sporadic variants) are quite rare.

G6PD gene is located in the long arm of chromosome Xq28 and includes 13 exons and 12 introns with the length over 20 Kb (Beutler, 1994; Nguyen et al., 2008; Noori-Daloii et al., 2006). The enzyme monomer consists of 515 residues with over 59 kDa in calculated molecular weight (Beutler, 1994; Luzzatto and Poggi, 2008). It was
reported that the enzymatically active form of G6PD is either a dimer or a tetramer of a single polypeptide subunit according to pH of cellular environment (Cohen and Rosemeyer, 1969; Luzzatto and Poggi, 2008; Naylor et al., 1996). Each subunit includes two main domains: a classic dinucleotide-binding fold (residues 1 to 198) and the β + α fold (residues 199 to 515). The classic dinucleotide-binding fold contains the coenzyme binding site while the β + α fold contains the substrate binding site (199 to 205 aa), a site for a structural NADP+ molecule [487 to 515 amino acids (aa)] and a surface forming the dimer-interface (380 to 425 aa). The coenzyme binding site and the substrate binding site have essential functions in the efficiency of G6PD enzyme; most severe diseases are caused by mutations at the structural NADP+ binding site and the dimer interface in which the components are contributing to the enzyme stabilization. According to the G6PD mutations database of Minucci et al. (2012), majority of class I mutations exhibit their effect on the exons 6, 10 and 13 encoding the substrate binding site, dimer interface and structural NADP+ binding site, respectively. In addition, 108/186 point mutations are located on the regions (257 to 515aa) encoded by exons 8 to 13 and 66/74 class I mutations occur in there (Minucci et al., 2012).

Due to its serious effects on human health, many researches have been carried out in other to understand as much as possible G6PD deficiency. Nowadays, the G6PD tests based on the molecular techniques are expected to overcome the limitations of biochemical tests because of the stability of DNA. These diagnostic techniques work on DNA sequence, so they can detect the heterozygous females with higher sensitivity than protein methods (11 to 32%) (Cohen and Rosemeyer, 1969). Moreover, the diagnostic methods based on DNA can reduce the number of the false negative or false positive results in biochemical tests that can be created by some other factors such as: reticulocytes, white blood cells, age of erythrocytes and temperature. Besides that, to develop the molecular tests, studies about causative mutations in each population are very necessary because the causative mutations and their frequency are extremely different in each population. For example, the Mediterranean (563C>T) is the most common mutation in Southern Europe while A- (202G>A, 376A>G) is particularly prevalent in Africa, and Viangchan (871G>A) is most popular in South-East Asian (Naylor et al., 1996). Consequently, the result from the mutation screening among each population will be a useful tool in the G6PD deficiency molecular database establishing for that population and a precondition for the molecular test developments.

In Vietnam, G6PD deficiency has been studied since 1960s and the general incidence is about 9.7%. However, the prevalence varies according to the ethnic groups and regions. In the north, its prevalence ranges from 0.5 to 31% (Verle et al., 2000) whereas it is just about 1.9 to 4.4% in Southern Vietnam (Matsuoka et al., 2007). The prevalence of G6PD deficiency is also diverse in different ethnic groups of Vietnamese population. For instances, the frequencies are 0.5 and 0.7% with Kinh and Mong population in the Northern, respectively (Verle et al., 2000) while the one of Kinh in Lam Dong province is 1.93% (Matsuoka et al., 2007). Recently, some research groups investigated about mutations on G6PD gene in Vietnamese population and revealed some valuable conclusions. Matsuoka et al. (2007) detected seven mutations in 25 cases by direct sequencing. Hue et al. (2009) found out six mutations in Kinh and Stieng’s ethnic population by SSCP. Not many studies about G6PD mutations in Vietnamese were performed. The common and main causative mutations of G6PD deficiency in Vietnamese were not found.

In the purpose of development of a molecular diagnostic test for G6PD deficiency in Vietnamese-kinh population, majority in Vietnam, to overcome the limitation of recently methods, this study was conducted to detect the common mutations occurring in the G6PD gene which cause G6PD deficiency in Vietnamese-kinh population.

MATERIALS AND METHODS

Subjects

Dried blood spot samples were collected from Vietnamese-Kinh G6PD deficient male newborns, in the Tu Du Maternity Hospital - Ho Chi Minh City. The G6PD deficiency is identified by enzyme level lower than 5.1 IU/gHb, using the neonatal G6PD screening assay, an enzymatic colorimetric assay, provided by the manufacturer (Zentech, Belgium). The principle of this assay is based on the tetrazolium salt method (Van Driel and Van Noorden, 1999). Three blood spots were collected from each newborn and one of them was used for measuring enzyme activity while the others two blood spots were used for DNA extraction.

Thirty (30) samples were used for detection of mutation using sequencing. Within 30 G6PD deficient patients, 27 patients with enzyme level lower than 5.1 IU/gHb were collected. One of them had enzyme level of 5.3 IU/gHb; this case has enzyme activity a little bit higher than the cut-off but the clinical symptoms are clear for G6PD deficiency. Two patients had no data of enzyme level but with clear symptoms. This indicates some limitation of the colorimetric assay. All samples were collected with the consent form signed by newborns’ parents. Figure 1 shows the distribution of samples within the cut-off of enzyme activity level. Only one sample had enzyme activity level higher than the cut-off of 5.1 IU/g Hb.

Another group of samples were used for confirming the frequency of the most common missense mutations in the G6PD deficient Vietnamese-kinh. This group of sample contained eight females and 129 males. All samples were confirmed for G6PD deficient clinical symptoms and enzyme activity level lower than 5.1 IU/g Hb. The ratio between female and male patients was about 1:16.

DNA extraction

Human genomic DNA was extracted from dried blood samples using phenol: chloroform: iso-amyl = 25:24:1 according to the two-
step lysis method. This method was improved from method of Chaisomchit (Chaisomchit et al., 2003) and reported as a quite effective way for extracting DNA from dried blood sample (Hue et al., 2011). DNA quality and quantity are measured by ultraviolet (UV)-nanodrop 1000 spectrophotometer machine.

**Primers and DNA fragments design**

For identifying mutations that occurs within coding region of G6PD gene, the sequencing will be performed. Before sequencing, the DNA fragments from G6PD gene should be amplified. With 12 exons spent across 13 kb, the primers for amplification and sequencing were designed focusing on the exons region only. The primers were intended to design amplifying DNA fragments with the size from 200 to 900 bp which will give the best results in sequencing by 3130xl genetic analyzer (Applied Biosystems, France). Primer3plus software and BLAST tool in NCBI website were used for designing primers. The designed primers were then sent to Sigma Aldrich- Singapore for synthesis.

**DNA fragments amplifying**

PCR were performed on the Eppendorf® PCR machine. Each 25 µl PCR reaction included 1 µl of specific primers 10 µM, 12.5 µl TopTaq Master Mix 2x, around 20 ng of DNA template and added dH2O. The optimized PCR cycling conditions were as follow: one step of initial denaturation (94°C for 3 min); followed by 40 cycles of 94°C for 30 s, annealing temperature (Ta) for 30 s, 72°C for 1 min; and a final extension period at 72°C for 5 min. PCR products were assessed by running the electrophoresis on a 1.5% agarose gel, containing 0.5% ethidium bromide with the voltage of 70 v in 20 min; then light bands were observed under UV light (using the UVP GelDoc-It®Imaging system).

**DNA sequencing**

PCR products were purified by the QIAquick PCR purification kit, and then sequencing reactions were performed for both strands of DNA by using ABI prism BigDye terminator cycle sequencing ready reaction kit and the sets of primers that were similar to the others used for fragments amplification. These sequencing reactions were run on the 3130xl Genetic Analyzer (Applied Biosystems, France). The thermal cycle sequencing performed on the previous 20 µl mixture with the temperature program was as follow: 96°C for 1 min, and then 25 cycles with 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. After thermal cycling, the extension products were re-purified to remove excess dye terminators prior transfers to capillary electrophoresis. The results were transferred to PC through the detector by capturing signal emitted from the dye terminators. The DNA sequences were analyzed by SeqScape v2.5 software. The sequences of fragments were compared to the sequence of G6PD gene in Ensemble (accession No.ENS00000160211) to identify the mutations.

**RESULTS**

Seven pairs of primers were designed to fragment the G6PD gene into seven fragments with the size from 241 bp for the smallest fragment to 882 bp for the largest fragment (Table 1). Some primers were designed to amplify two exons next together in one fragment, and some parts of intron were also included in the fragment also (Figure 2).

With 30 DNA samples extracted from 30 blood samples and 7 sets of primers, 210 PCR products were amplified then purified for sequencing to identify mutations. There were seven different point-mutations detected in G6PD gene in Vietnamese-Kinh G6PD patients (Table 2). Two mutations which occurred within the exons with high frequency are Viangchan (871 G>A) and Canton (1376 G>T) with 43.33 and 26.6%, respectively. A silent mutation (1311 C>T) occurred in an intron of the gene with frequency of 56.66%. Four other mutations occurred within exons of the gene with lower frequency are Union,
Table 1. PCR primers for amplifying and sequencing 7 fragments including 12 exons of G6PD gene.

<table>
<thead>
<tr>
<th>Name</th>
<th>Primer sequence</th>
<th>Exon</th>
<th>Size (bp)</th>
<th>Ta (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1-F</td>
<td>5'-CTCAAGAAAGGGCTAACTTCTCTCA-3'</td>
<td>2</td>
<td>241</td>
<td>63</td>
</tr>
<tr>
<td>F1-R</td>
<td>5'-GCACCTCCTGCTTTAAGATTGGG-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2-F</td>
<td>5'-AGGTCGTTCCCAGCCACT-3'</td>
<td>3 and 4</td>
<td>641</td>
<td>66</td>
</tr>
<tr>
<td>F2-R</td>
<td>5'-GCAGACACTGCCCCAGGC-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3-F</td>
<td>5'-GGGCACCTCCCTGGAACCTC-3'</td>
<td>5</td>
<td>491</td>
<td>65</td>
</tr>
<tr>
<td>F3-R</td>
<td>5'-TCGTGGAGCAAGCTGTGCCAC-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F4-F</td>
<td>5'-ACTCCCCGAAGAGGGTCAAGG-3'</td>
<td>6 and 7</td>
<td>561</td>
<td>63</td>
</tr>
<tr>
<td>F4-R</td>
<td>5'-GCTCTGCCACCCTGTGCCAG-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F5-F</td>
<td>5'-ACACAGCCAAGCACCACCA-3'</td>
<td>8</td>
<td>607</td>
<td>65</td>
</tr>
<tr>
<td>F5-R</td>
<td>5'-CAGGGCCCTCCCCTGAGGAC-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F6-F</td>
<td>5'-CAAGGAGCCATTCTCTCCCT-3'</td>
<td>9, 10 and 11</td>
<td>882</td>
<td>62</td>
</tr>
<tr>
<td>F6-R</td>
<td>5'-CCCCATAGCCCACAGGTATGCAGG-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F7-F</td>
<td>5'-TGTTGCAACCGGCTCACA-3'</td>
<td>12 and 13</td>
<td>661</td>
<td>69</td>
</tr>
<tr>
<td>F7-R</td>
<td>5'-GTCTCACTCTTGAGGGGCA-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Designed amplicons and their locations on G6PD gene. F1- F7, designed amplicons; E1-E13, position of exons in the gene.

Kaiping, Gaohe and QuingYuang with frequency from 3.33 to 6.66% (Table 2).

The discovered mutations were located in exons 9, 11, 12 while no mutations was found in exons 3, 4, 6, 7, 10 and 13 (Table 2, Figure 3). The patients with clinical symptom of G6PD deficiency contained at least one mutation within G6PD gene even the silent mutation; in which 40% (12/30) of patients contain two mutations and 3% (1/30) of patients contain three mutations.

Even in small sample size of 30 patients, 56.66% of patients had silent mutation 1311C>T, in which 23.5% appeared alone and 76.5% appeared with another
Table 2. G6PD variants have been detected in the coding region of G6PD gene in Vietnamese-Kinh G6PD deficient patients.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Mutation position</th>
<th>Substitution</th>
<th>Class</th>
<th>Exon</th>
<th>Frequency (%)</th>
<th>Enzyme activity (IU/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaohe Ghaozhou</td>
<td>95 A&gt;G</td>
<td>32 His&gt;Arg</td>
<td>III</td>
<td>2</td>
<td>3.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Chinese-4 Quing Yuan</td>
<td>392 G&gt;T</td>
<td>131 Gly&gt;Val</td>
<td>III</td>
<td>5</td>
<td>3.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Viangchan</td>
<td>871 G&gt;A</td>
<td>291 Val&gt;Met</td>
<td>II</td>
<td>9</td>
<td>43.3</td>
<td>1.1 - 3.5</td>
</tr>
<tr>
<td>Union Chinese-2</td>
<td>1360 C&gt;T</td>
<td>454 Arg&gt;Cys</td>
<td>II</td>
<td>11</td>
<td>6.6</td>
<td>0.7 - 1.4</td>
</tr>
<tr>
<td>Canton</td>
<td>1376 G&gt;T</td>
<td>459 Arg&gt;Leu</td>
<td>II</td>
<td>12</td>
<td>26.6</td>
<td>1.9 - 2.2</td>
</tr>
<tr>
<td>Kaiping</td>
<td>1388 G&gt;A</td>
<td>463 Arg&gt;His</td>
<td>II</td>
<td>12</td>
<td>6.6</td>
<td>3.6 - 5.3</td>
</tr>
<tr>
<td>Silent mutation</td>
<td>1311 C&gt;T</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>56.6</td>
<td>0.7 - 5.3</td>
</tr>
</tbody>
</table>

Figure 3. The detected mutations and their location on the G6PD gene.

Table 3. Genotype and allele frequency of Viangchan mutation in the G6PD deficient patients.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Female (n=8)</th>
<th>Male (n=129)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Allele frequency</td>
<td>G</td>
<td>A</td>
</tr>
</tbody>
</table>

*Y=Y chromosome in male; it do not contain desire allele.

mutations. All of the patients containing more than one mutation had the silent mutation, 1311C>T. The linkage between the silent mutation 1311C>T with Union or Kaiping occupied 16.7% (2/12); while the linkage with Canton is 66.7% (8/12) and with both Gaohe and Quing Yuan was 8.3% (1/12). While most mutation show the linkage disequilibrium with 1311C>T, the most common missense mutation Viangchan had no linkage neither others nor 1311C>T.

To confirm the common Viangchan mutation in Vietnamese-Kinh population, the genotyping was performed to identify the frequency of Viangchan alleles. The result show that the mutant allele (A allele) of Viangchan mutation occupied 33.8% in the patient population. 35% Vietnamese-Kinh G6PD deficient patients caused mutant allele of Viangchan mutation (GA + AA + YA) (Table 3).

DISCUSSION

In the purpose to find out the most common mutation occurring in Vietnamese Kinh patients, the screening mutation was carried out in G6PD deficient male newborns only because this is a recessive X-linked disease; normally the symptoms are clearly seen in male but not in female, particularly when female contain only one mutant allele. Thus, the male newborns were selected for this study to make sure the symptoms are related to the G6PD deficiency but not others. The ratio of 1:16 between male and female patients is also a reason to select male only for the mutation screening. By
this selection, the sequencing was performed for both DNA strands of each person bring more evidence for confirming the appearing of mutations within the DNA fragments.

In the previous studies, there were many mutations detected and were demonstrated as the common mutations in Southeast Asia area. Canton and Kaiping mutations were the most prevalent in Chinese population in Southern China (40 to 75%), Taiwan (76%), Malaysian Chinese (84%) and Singapore Chinese (45%) (Laosombat et al., 2005); Viangchan is the dominant mutation in South–East Asia with frequency from 9 to 88.9% (Matsuoka et al., 2007; Nguyen et al., 2009). In Lao and Cambodia, Viangchan occupies more than 90% of G6PD-deficient cases (Iwai et al., 2001; Louicharoen and Nuchprayoon, 2005), and it obtains 31.35% in Thailand (Laosombat et al., 2005). Located in the Southeast Asia, Vietnam should share the same common mutations with other countries in the same region. In addition, Vietnam is the continent of China for thousand years; it is also the reason for Vietnamese sharing the same mutation patterns with Chinese. Among seven mutations that have been detected on G6PD gene in this study; five mutations (Gaohe, QuingYuan, Union, Canton and Kaiping) have been described in Chinese population and the other two mutations (Viangchan and 1311C>T) are described in Southeast Asia populations. This finding shows the evidence for the geographically distribution of causative mutations of G6PD deficiency on over the world.

However, some mutation such as mutation Mahidol (487G>A), the most important mutation in Thai, Indochina peninsula and Myanmar (Iwai et al., 2001; Matsuoka et al., 2007), was not found in Vietnamese population in this study. It could be due to the fact that the frequency of this mutation is low in Vietnamese and cannot be found in this small sample size. In previous studies, some novel mutations were detected in Vietnamese population such as Vietnam1 (3Glu >Lys); Vietnam2 (66Phe >Cys); Vietnam3 (73Ser >Ser) (Hue et al., 2009) and Bao Loc (118 Tyr > His) (Matsuoka et al., 2007), but in this study they were not detected. This may be because the studies were performed in different ethnic population in Vietnamese. This study was only performed in Vietnamese-kinh ethnic while the previous study was performed in several ethnicities in Vietnamese. Those novel mutations may occur only in minor ethnic while in Kinh they did not exist. Thus, even though the causative mutations of the disease are geographically distributed, the most common or the main causative mutations may be specific for each population or ethnicity. In the purpose of finding the common mutation cause of the disease, the low frequent mutations may be ignored as the diagnostic test could be developed based on only the most common mutations for common used and detect most cases. In this study, Viangchan was found as the most common mutation in Vietnamese-kinh. This mutation have also been demonstrated as the common mutation in Chinese, Southeast Asia and even Vietnamese in the previous study (Laosombat et al., 2005; Matsuoka et al., 2007; Nguyen et al., 2009; Hue et al., 2009). Thus, even with the small sample size, the finding that Viangchan is the most common missense mutation in Vietnamese-kinh, in this study, is adequate enough, which is proposed for further study after that. Genotyping Viangchan mutation in a bigger sample size showed the common mutation in the population. This indicates that if Viangchan mutation is used as the marker for diagnosis of the disease, at least 35% patients will be detected. The other common mutations such as Canton and 1311C>T could be used as the markers for diagnosis even fully understanding about the function has not been shown. The use of these mutations as the marker may lead to the best diagnostic test as its possibility in detection of the disease reach to more than 90% (1311C>T occupied 56.6% and Viangchan occupied 35% in the population).

To evaluate the possibility to use those mutations as the diagnostic markers, beside the high frequency in the population, the linkage between them needs to be analyzed. As the most common mutation in Vietnamese, the Viangchan mutation had shown the linkage disequilibrium with silent mutation 1311C>T in Vietnamese-Stieng’s population (Hue et al., 2009), and in Thai and Malaysian (Ainoon et al., 2003; Iwai et al., 2001; Nuchprayoon et al., 2002). However, in this study, Viangchan and 1311 C>T did not show the linkage disequilibrium in Vietnamese-Kinh, while in addition, the 1311C>T is known to be linked to Mediterranean 563 C>T mutation in Palestinians (Sirdah et al., 2012a), in this study, it linked to other mutations such as Canton, Kaiping, Union, Gaohe, and QuingYuang. 1311C>T appeared in many populations and with high frequency; it shows the linkage to other mutations in different pattern. Thus, it could be the universal marker for G6PD deficiency while other mutations can be used in the combination for the special cases. A set of diagnostic markers for each population should be different. In Vietnamese-kinh, the Viangchan appeared with high frequency and not linked to other mutations; it can be used as diagnostic marker for Vietnamese-kinh. Besides that, the combination between 1311C>T and Canton may also be the potential markers. However, the function and the association between these mutations with the disease need to be clarified.

Six detected mutations in this study are classified to the class II and III (WHO). With these classes, the symptom of the disease is not very serious. This can be explained by the position of the mutation in related to the position of amino acid in the protein structure. The G6PD protein has two main domains: the small domain contain amino acid 1 to 198 in the polypeptide, with the coenzyme binding site from amino acid 38 to 44; the large domain including amino acid 199 to 515, with the substrate binding site from amino acid 199 to 205. In the structure of G6PD
protein, the site for binding a molecular to NADP is from amino acids 487 to 515, and the dimer-interface site is from amino acids 380 to 425. In theory, the mutations occur in the large domain, the interface sites or the binding site share a strong effect on the activity of the enzyme as it affects the functional structure of the protein. The detected mutations in this study are located on amino acid 32, 131, 291, 437, 454, 459, 463 which are not included in the coenzyme binding site, the substrate binding site or the dimer interface, thus they do not affect the activity of the protein much. They can cause reduction of the enzyme or activity of the enzyme; but not very severe. That explains why the symptom of G6PD deficiency disease in Vietnamese is not very severe and only classify as class II or III (WHO classification).

As the highest frequency mutation, 1311C>T located in the intron of the gene where shown no affecting to the protein structure or enzyme, but it related to the low G6PD activity level (0.7-3.7 IU/g Hb) in this study. Thus, how does it affect the enzyme activity and lead to the disease? In theory, the low level of enzyme activity may cause lost or reduce interaction between monomers of enzyme that lead to no function of enzyme in the blood, or by lacking the interaction with the substrate. In anyway, the mutations which lead to the changing in the protein structure can cause low enzyme activity level. But the mutation 1311C>T is located in the intron of the gene; it does not affect the structure of protein. The low enzyme activity, in this case, may not directly correlate to the protein structure changed but the regulation of transcription of the gene leading to the reducing of enzyme level. This silent mutation may be located in the enhancer region which helps to increase the level of enzyme expression. Thus, when the mutation occurs, it destroys the function of the enhancer, leading to the reduction of the enzyme level. The silent mutation 1311C>T may also relate to a microRNA, which regulates the G6PD gene expression. The mutation occurred leading to the activation of microRNA, and then inhibiting the gene expression. Thus, even though the 1311C>T mutation is a silent mutation and locate in the intron region, it had shown the correlation with low enzyme level, so it may be the good candidate marker for a molecular diagnostic test.

Another point seen in this study was that the fluorescence spot test is not the accurate test for diagnostic of G6PD deficiency. There was a patient who had two mutations, Kaiping and 1311C>T with normal enzyme activity level (5.3 IU/g Hb), while another patient had the same mutations showing the abnormal level of the enzyme (3.6 IU/g Hb). In addition, two samples with no information about enzyme level due to some problems in the fluorescence test were identified containing mutation also. Thus, the detection of mutations could be that the molecular test support the current test in some inadequate cases. By the fluorescence spot test, only the severe cases can be detected correctly while other cases and carriers cannot be detected. By finding the common mutations in this study, a molecular diagnostic test can be developed to diagnose the G6PD deficiency in Vietnamese.

Conclusion

Seven different point-mutations were detected in G6PD deficient Vietnamese. Viangchan is the most missense mutation and 1311C>T is the most common silent mutation. The next common mutation that also needs to be cared about is Canton as it has the linkage with 1311C>T. Certainly, the presence of missense mutations in G6PD gene cause low enzyme activity, however, the silent mutation 1311C>T was also related to the low enzyme activity level. As the most common mutations in the Vietnamese-kinh population and their linkage disequilibrium, the Viangchan, Canton and 1311C>T could be used as the diagnostic markers for the detection of G6PD deficiency in patients in Vietnamese-kinh.

ACKNOWLEDGEMENTS

This study was funded by National Foundation for Science and Technology Development (NAFOSTED). The authors would like to thank all patients who agreed to participate in this study by giving their blood as samples, thanks to Dr. Nguyen Khac Han Hoan, Dr. Huynh Thi Thu Thuy and staffs at Tu Du Maternity Hospital for the help in the sample collection.

REFERENCES


Laosombat V, Sattayasevana B, Janejindamai W, Viprakasit V, Shirakawa T, Nishiyama K, Matsu M (2005). Molecular heterogeneity of glucose-6-phosphate dehydrogenase (G6PD) variants in the south of Thailand and identification of a novel variant...


