THE STUDY OF GLUCOSE- 6 - PHOSPHATE DEHYDROGENASE (G6PD) GENE BY RESTRICTION ENZYME DIGESTION IN THE KELANTAN POPULATION

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Abstract

The most common diseases producing enzymopathy affecting the human population is glucose-6-

phosphate dehydrogenase (G6PD) deficiency. It is estimated that about 400 million people

worldwide are affected and this disease is commonest seen in the tropical and subtropical zones of

the Eastern hemisphere. Molecular analysis has confirmed that the basis for G6PD deficiency is

widely heterogeneous. Different mutants are responsible for the G6PD deficiency in the various

parts of the world where this abnormality is prevalent. This study involved a sequential analysis

whereby the blood from Malay neonates with neonatal jaundice admitted to Hospital Universiti

Sains Malaysia and Kota Bharu Hospital were analyzed and polymerase chain reaction based

analysis using serial multiplex primer method was done on those DNA samples. Samples that are

found to be abnormal were then sequenced. Out of the 45 samples studied, 8 were found to have

the Mediterranean mutation, two have the Mahidol mutation, two have Canton mutation and three

have Kaiping mutation. Thus the molecular basis for the Malay neonatal jaundice in Kelantan is

described with further prospect of population screening.

INTRODUCTION

The most common disease-producing enzyme deficiency affecting the human population is glucose-6-phosphate dehydrogenase (G6PD) making it the most common metabolic disorder of the red blood cells. G6PD is important in the metabolism of glucose especially so in the red blood cells whereby its role is in maintaining a high cellular level of NADPH. NADPH is required for reductive biosynthetic reactions and for defense against oxidative stress. The only means of providing NADPH is by G6PD and without this the red blood cell may be susceptible to damage especially in G6PD deficient individuals.

Studies on G6PD variants at the molecular level during the last ten years have undermined more than fifty variants in all parts of the world where this disease is common including South East Asia. Exon 6,7,10,11 and 12 of the G6PD gene are subject of interest for mutation studies since the candidate mutations are postulated to be here based on population background, clinical features and presentation.

Kelantan is one of the 13 states in the northern part of Malaysia, with South of Thailand as the north border of Kelantan. The population of Kelantan is estimated to be 1 million and the majority are Malays with Chinese and Indian as minority.

In Kelantan G6PD deficiency is a common cause of neonatal jaundice and screening for this disease has been done in the newborns since the last 10 years by UV fluorescent screening method. Beside neonatal jaundice which can be severe, favism is also described in these G6PD deficient individuals.

METHODOLOGY

Biochemical assay

Biochemical assay for G6PD were done using the kinetic determination assay (Sigma. USA).

Samples collection and DNA extraction

DNA samples from babies with neonatal jaundice confirmed as G6PD deficiency by enzymatic ultraviolet fluorescence technique were extracted from whole blood using the standard phenol extraction method.

First PCR amplification

The exon 6 encompassing region of the G6PD gene was amplified by using primers on intron 6 and 7. The sequence of the primers was as described before (Hirono et al. 1994). PCR was performed in a 10 -20 ul reaction volume using the Perkin Elmer Thermocycler with the following cycles: 30 cycles for 1 min at 94^0 C, 1 min at 58^0 C, 1 min at 72^0C followed by 1 cycle at for 10 min with Primers 6.7-1 and 6.7-2 (see appendix). PCR products then underwent electrophoresis using a 2% agarose gel containing 0.5 ug/ml ethidium bromide with 100 bp DNA size marker. If the density of the amplified products was not as dense as the DNA marker supplementary PCR was performed with the same PCR condition using primers 6.1 and 6.2.

Second PCR amplification

In the second PCR each of the forward primers was designed to work with one common inverse primer. To cover the region of exon 6 3 forward primers in tandem ranging from 11 to 15 mer overlapping each other with 3 to 5 nucleotides and one common inverse or reverse primer were used.

PCR was carried out using the hot start method. 10 ul of pre- mixture A containing MPTP master mixture, 1 ul of the primer, 1 ul of the PCR product were then mixed with Pre-mixture B containing 8 ul of MPTP master mix, 1.9 ul distilled water and 0.1 ul of 5U Amplitaq DNA polymerase Stoffel Fragment (Perkin Elmer) were preheated and mixed at 95^0C. Amplification is then continued at 95^0C, 47^0C and 30^0 C for 30 seconds for each cycle and the total cycles is 25. PCR products are subjected to electrophoresis on 4 % gel with metaphor gel.

DNA Sequencing

The exon 6 encompassing region of the G6PD gene was amplified from all DNA samples and subjected to direct sequencing using as dye terminator and the auto sequencer Perkin Elmer ABI Prism 310 Genetic Analyzer.

RESULTS

Biochemical assay on all these samples revealed that the level of G6PD enzyme in all the patients is severely deficient.

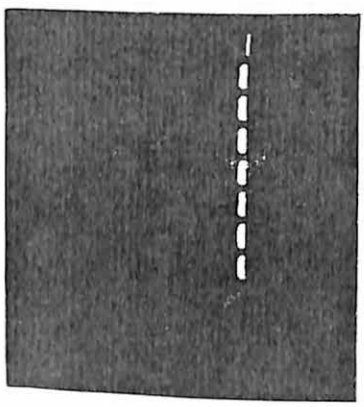

Fig. 1. First amplification of DNA samples from G6PD deficient neonates. Lane 1. Molecular marker. Lane 2-7. Part of DNA samples after the first PCR amplification showing satisfactory products. These samples are subjected further for the 2nd PCR amplification (MPTP) using different primers

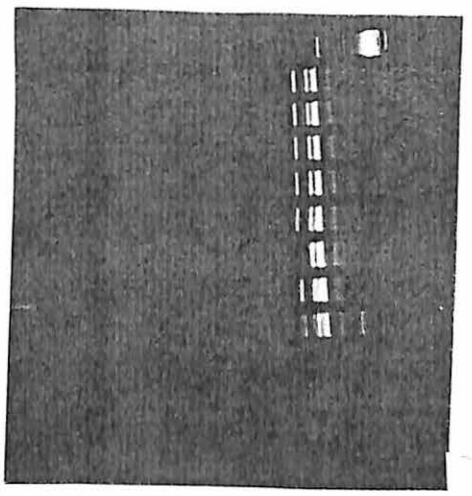

Fig. 2 MPTP amplification results of 8 patients. Lane 1. 50 bp marker. Lane 2,3,4,5,6,8. Lane 9 is normal control. Lane 7 shows the Mediterranean mutation.

For summary of all the DNA analyzed please refer to Table G6PD Variants in Kelantan

DISCUSSION

Out of the 45 samples of neonatal jaundice with G6PD deficient obtained 8 (17.8%) showed having Mediterranean mutations 563 C->T, two patients (4.4%) have the Mahidol mutation 487 G->A,

2 patients (4.4%) have Canton 1376 G>T mutation and 3 patients (6.7%) have Kaiping 1388 G>A mutation.

G6PD Mediterranean was first described in the people in the Mediterranean countries but since

then have been described in other parts of the for example India, United Kingdom and Singapore. Perhaps this explain the migration theory of the population throughout all parts of the world. G6PD Mahidol mutation is the predominant variant in Thailand and the results that we get is not surprising due to our close proximity to Thailand perhaps through intermarriages. This theory also explains for the other two mutations found.

With an high incidence of neonatal jaundice (2.5% per year) we expected that this mutation might be common in this part of the region thus demonstrates the underlying neonatal jaundice and defect in an individual.

MPTP as a tool for mutational analysis is found to be fast and accurate to screen a patient mutation because the use of short primers and amplification of several fragments in one reaction using 3 sets of primers. There is no misannealing of primers because the size are only 11 -15 base pair in length.

MPTP is an excellent tool for mutation analysis and population screening in a region where G6PD deficiency is frequent and a spectrum of specific mutations are known.

For the future we would like to characterize the other DNA that have not been done yet. It could be due to the fact that the other exons that is exons 2,3,4,5 that may contain the mutation or perhaps due to a new mutation that have not described elsewhere before and which is specific to the people of Kelantan. Thus this could be the aim of our next study.

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45 G6PD Variants in Kelantan.

DNA No.	SMP					SEQUENCE	VARIANT	REMARKS (WHO Class)
	Exon 6	Exon 7	Exon 10	Exon 11	Exon 12			
1								
2								
3								
4								
10								
11					Abn	1376 G>T	Canton	2
15	Abn			Abn (sil)		563 C>T	Mediterranean	2
16								
17	Abn					563 C>T	Mediterranean	2
21	Abn					563 C>T	Mediterranean	2
23								
24								
25					Abn	1376 G>T	Canton	2
26								
28				Abn (sil)				
30								
31	Abn			Abn (sil)				
32								
33								
34				Abn (sil)				
35				Abn (sil)				
36								
37	Abn					563 C>T	Mediterranean	2
38				Abn (sil)				
39				Abn (sil)				
40	Abn					563 C>T	Mediterranean	2
41					Abn	1388 G>A	? Kaiping	2
42	Abn					563 C>T	Mediterranean	2
43	Abn					487 G>A	Mahidol	3
44								
45								
46	Abn							
47								
48				Abn (sil)	Abn	1388 G>A	? Kaiping	2
49				Abn (sil)				
50				Abn (sil)				
52								
51				Abn (sil)				
53	Abn					563 C>T	Mediterranean	2
54					Abn	1388 G>A	? Kaiping	2
55								
56	Abn					487 G>A	Mahidol	3
57						563 C>T	Mediterranean	2
58								
59								

Keys:

sil: **silent mutation**

Abn: **Abnormal**