Perspectives of Glucose-6-Phosphate Deficiency-Overview

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) deficiency which is a genetic disorder, it is an X-linked hereditary defect caused by the mutations in the G6PD *gene. G6PD deficiency is one of the most prevalent human enzymopathies affecting more than 400 million individuals worldwide, and particularly in those undeveloped and the resource-limited countries. The clinical phenotype of G6PD deficiency varies significantly from the asymptomatic to neonatal jaundice, kernicterus, or the acute hemolytic anemia following the ingestion of certain drugs during some infections, and notably through eating the fava beans (favism).*

KEYWORDS: *Glucose-6-phosphate dehydrogenase (G6PD), deficiency, enzymopathies, hemolytic anemia, X-linked hereditary, fava beans (favism).*

INTRODUCTION

This variability in the clinical phenotypes has been attributed to diverse mutant types in the *G6PD* gene. Till date, > 180 mutations have been reported and recorded worldwide, and each ethnic population presents a characteristic mutation spectrums. For example, in the Chinese population, at least 21 different mutations have been allied with G6PD deficiency. These mutations cause class II (much severe) or class III (mild) deficiencies, in which anemia is not present in daily life, but the hemolytic attack can occur upon ingestion of certain oxidative medicines or foods . Therefore, screening for the affected individuals is critical for prevention of the disease (Gómez-Manzo et al., 2016a).

Biochemical assays based on the G6PD-catalyzed production of nicotinamide adenine

dinucleotide phosphate (NADPH) are widely availed for new born screening (Gómez-Manzoet al., 2016b). Despite the success in identifying male patients, measurement of the G6PD activity appears to be inadequate for the detection of heterozygous females due to the lyonization (inactivation of one X chromosome). To overcome this limitation, many alternative biomolecular assays have been developed, encompassing denaturing high-performance liquid chromatography (DHPLC), amplification refractory mutation system (ARMS), the microarray-based assay and the reverse dot blot assay (RDB). Although each assay has unique advantages in terms of specificity and the sensitivity, a common shortcoming of these methods is that they often involving multiple steps of the post-PCR manipulations, which increase the technical complexity and the risk of amplicon contamination. The High-resolution melting (HRM) is a good choice to obviate the post-PCR ramification; nevertheless, the performance of the dye-based methods is compromised by an inability to precisely identify the mutations (Flores et al., 2017).

The MeltPro G6PD (Zeesan, Xiamen, China) assay is a precisely qualitative diagnostic assay developed based on multicolour melting curve analysis (MMCA) availing dual-labeled, self-quenched probes. This bioassay was designed to detect the genotypes of 16 mutations in the *G6PD* gene, which covers > 95% of the Chinese *G6PD* mutations. The Melt Probioassay is a closed-tube format performed on a real-time PCR platform, which is a precise bio technique from which the mutation information is retrieved based on differences in melting temperature (ΔT_m) compared to the wild-type. One distinct or unique feature of this bioassay is its ease-of-use due to the omission of complex post-PCR manipulations. Moreover, the exact mutations type can be identified based on the predefined T_m values and the detection channels (Desforges et al., 1991).

In this study, researchers systematically evaluated the analytical and clinical performances of the MeltPro G6PD assay. For the analytical research, the accuracy of mutation detection, the limit of detection, the reproducibility, and the cross-platform biocompatibility were evaluated. For the clinical study, a multicenter validation study was performed availing 763 clinical samples collected from three different hospitals in China. Researchers examined both G6PD enzyme activity results and DNA sequencing results.

EXPERMENTAL BASIS

Glycolysis-deficient mutants from the Chinese hamster cells have been used to investigate the role of glycolytic enzymes in the uptake and the metabolism of sugars and the role of insulin receptors and the insulin-dependent kinase reactions in control of metabolism (Chen et al., 2008). One such mutant, designated 'RI.1.7', is deficient in the two enzymes of glycolysis: glucose phosphate isomerase (GPI) and the phosphoglycerate kinase (PGK). As a consequence of these defect, R 1.1.7 cells, unlike the parental (CHO-K1) cells, do not grow in media comprising mannose as sole carbon source (Carson,1971) and mannose is, in fact, toxic Revertants of RI.1.7 now able to precisely grow in mannose media and resistant to mannose toxicity have been isolated. Such revertants are now also quiet deficient in hexokinase (Cappellini et al., 2008). Here researchers have used a hexokinase-deficient mutant of Chinese-hamster ovary (CHO) cells, M + R 42 (which is also deficient in the GPI and PGK), to investigate the role of the hexokinase in the control of 2-D-deoxyglucose (2-dGlc) transport and the accumulation within Chinese-hamster ovary cells.

In the preceding research, it was shown that:

- The 2-dGlc transport and accumulation are coupled to hexokinase activity in rat thymocyte;
- Simulation of thymocyte activation and accumulation of 2-dGlc transport by phorbol 12-myristate 13-acetate is accompanied by the tighter coupling of transport to the hexokinase activity.

The main evidence in favour of this view is that:

1. There is a phorbol-stimulated steady-state accumulation of the free 2-dGlc within the cytosol of thymocyte to a concentration 25-40-fold above the external concentration of 0.11mM;
2. net exit of 2-dGlc from the phorbol-activated cells after a period of accumulation of 2-dGlc is slower than from the control cells;
3. the high-affinity inhibitor of hexokinase, mannoheptulose, inhibits uptake of the 2-dGlc in both control and activated cells(Chiu et al.,2019);
4. Phorbol has no effect on exit of the 3-0- methyl D-glucoside (3-OMG), despite the fact that this non-metabolized sugar or the carbohydrate interacts with the 2-dGlc transporter.

These data are consistent with a model for active transport of the sugar where hexokinase activity at the cytosolic platform of the transporter transforms the transported 2-dGlc to impermeant hexose phosphate, thereby reducing the sugar reflux and creating an energy dependent rectifier for sugar movement across the membranes. The availability of the hexose-deficient CHO mutant is a useful and the unambiguous way of demonstrating linkage between hexokinase activity and transports (Ullrey et al., 1982). It is a prerequisite that the hexokinase-deficient mutant cells must have a fully competent sugar transporter.

In the present study researchers used wild-type and mutants CHO cells to demonstrate that:

- There is a phloretin-sensitive transport system for the 2-dGlc and 3-OMG in both wild-type and mutant cells of approximately the same activity;
- The hexokinase activity is deficient in the mutant cells;
- Free 2-dGlc is accumulated to a tissue: the medium ratio of approx. 10 in wild-type cells, but only by 1-2 in mutants;
- The exit of 2-dGlc is much slower from wild-type cells than from mutants; and
- No difference in the rate of the 3- OMG exit is seen between mutant and wild-type cells. The use of 2-dGlc, which is not a substrate for the Na+- dependent co-transport, precludes any possibility that this latter systems might obscure the interpretation of 2-dGlc accumulation.

Furthermore the absence of any bioaccumulation of 3-OMG by CHO cells indicates that the Na+ co-transport is absent from the system (Pouyssegur et al., 1980).

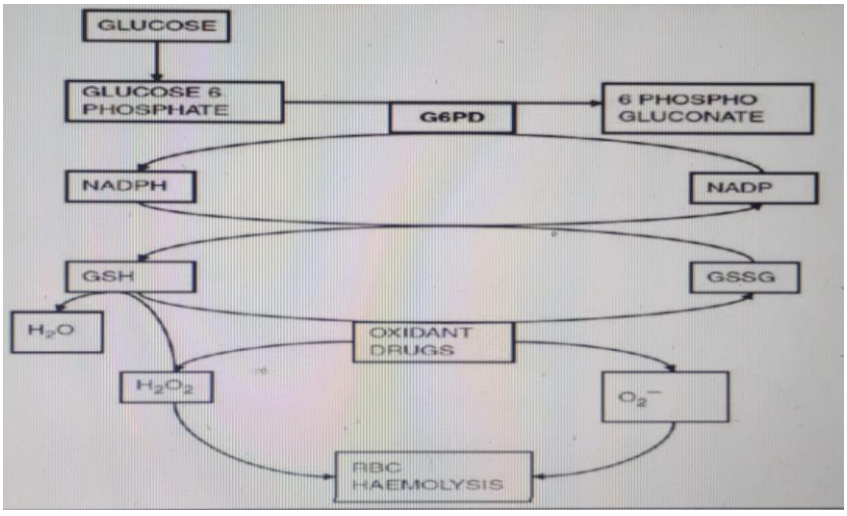

Fig 1: (Pathway)

The glycolytic pathway is one of the body's important and potent metabolic pathways. It involves a sequence of the enzymatic reactions that break down glucose (glycolysis) into pyruvate, creating the energy bio sources adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH). Various inherited defects in the enzymes of the pathway may occur (FIG-1) (Naftalin et al., 1989).

The most common defect is

- The Pyruvate kinase deficiency

Other defects that causes hemolyticanemia include deficiencies of

- Erythrocyte hexokinase
- The Glucose phosphate isomerase
- Phosphofructokinase

In all of these pathway defects, the hemolyticanemia occurs only in patients who are homozygous for the mutation. The exact mechanism of the haemolysis is unknown.

Symptoms are rakished to the degree of anemia and may include jaundice and splenomegaly. Spherocytes are absent, but the small numbers of irregularly shaped cells (echinocytes) may be present.

In general, bioassays of ATP and diphosphoglycerate help identify any metabolic defect and localize the defective sites for the further analysis. Genetic testing can also be performed (Naftalin et al., 1987).

TREATEMENT OF GLYCOLYTIC PATHWAY DEFECT

- Folic acid during the haemolysis process
- Transfusions if needed
- Sometimes splenectomy or spleen removal

Patients may require folic acid 1 mg orally once a day or transfusions. The effective dosage Iron chelation may be needed if there is evidence of iron overload.

For the pyruvate kinase deficiency, mitapivat, an oral activator of RBC pyruvate kinase, may be considered for adults with symptomatic anemia or transfusion dependence (1).

In severe cases, patients may be transfusion dependent, in which case, the splenectomy may be done. Hemolysis and anemia persist after splenectomy, although some improvement may occur, particularly in the patients with pyruvate kinase deficiency (Morgan et al., 198).

ABOUT GLU-6-P DEFICIENCY

Glucose phosphate dehydrogenasis (G6PD), the most prevalent enzymatic diseases in humans, exists in south-eastern Iran. The geographic correlation of its supply and distribution with the historic malaria endemic suggests that G6PD has aggrandized in frequency as a result of the natural selection by malaria. Based on studies, there is a controversy in terms of different analytical methods in terms of the resistance to malaria. Fifty malaria patients and 50 healthy individuals from several cities south-east of Iran were encompassed in the study and after obtaining consent, blood samples were taken from them. The G6PD enzyme deficiency was investigated using a fluorescent stain test (Faik et al., 1984).

DIAGNOSIS

The MeltPro G6PD bioassay is the first commercial genetic test for glucose-6-phosphate dehydrogenase (G6PD) deficiency. This multi colour melting curve bioanalysis-based real-time PCR assay is designed to the genotype 16 G6PD mutations prevalent in the Chinese population. Researchers comprehensively evaluated both the analytical and clinical performances of this assay. All the 16 mutations were accurately genotyped, and the standard deviation of the measured Tm was < 0.3 °C. The limit of the detection was 1.0 ng/μL human genomic DNA. The bioassay could be run on four mainstream models of real-time PCR machines (Morgan et al., 1977).

CASE STUDIES IN CHINA

Like most genetic disorders, the G6PD deficiency cannot be cured. However, Newborn Screening (NBS) is a simple and also cost-effective strategy to identify neonates at high risk of this disease, which precisely facilitates early diagnosis and timely intervention. Previous studies have demonstrated that G6PD deficiency is prevalent in parts of South China, such as Guangdong, Guangxi, Yunnan, and Hainan (Chen et al., 2008). Accordingly, NBS for G6PD

deficiency was initiated in South China, and is currently implemented in an extensive region of China due to its clinical importance for the diagnosis of the neonatal jaundice and haemolysis (Faik et al., 1977).

CONLUSION

G6PD deficiency it is a hereditary disorder, which is specifically an X-linked Heriditary disorder. This paper garners information regarding the different aspects of G6PD deficiency. The several bioassays conducted to diagnose G6PD deficiency.

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