Revision of WHO classification of G6PD variants and International classification of diseases (ICD)-11

Dr J. Cunningham and Dr. A. Bosman
Prevention, Diagnostics and Treatment
What is G6PD deficiency?

- X-linked, hereditary genetic defect due to mutations in the *G6PD* gene, causing functional variants with many biochemical and clinical phenotypes

- X-linked
- ~180 different mutations
- Mainly single base change

Drugs like 8 aminoquinolones create oxidative metabolites

Factors that can affect G6PD activity:
- G6PD variant – mutations variable stability
- Age of RBCs – older RBC more vulnerable
- Anaemia (malaria/Fe def)
- Hemoglobinopathies reducing RBC survival
- Reticulocytes resistance to oxidative stress
G6PD deficiency and *P. vivax* malaria

~ 400 million people affected

**FIGURE 2**

Endemicity of *P. vivax* in 2010

~ 7.5 million estimated in 2017

<table>
<thead>
<tr>
<th>Distribution in endemic countries</th>
<th>Q25</th>
<th>Median</th>
<th>Q75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele frequency</td>
<td>7.44%</td>
<td>8.04%</td>
<td>8.81%</td>
</tr>
<tr>
<td>G6PDd males</td>
<td>203,729</td>
<td>220,130</td>
<td>241,114</td>
</tr>
<tr>
<td>G6PDd females*</td>
<td>121,618</td>
<td>132,932</td>
<td>147,814</td>
</tr>
</tbody>
</table>
Relapses and vivax transmission

Battle KE et al., Adv Parasitol 2012; 80:1-111
8-aminoquinolines and G6PD deficiency

- Individual and public health risks posed by relapsing *P. vivax* should be taken into account when considering the risks and benefits of anti-relapse treatment.

Urine collection of a 5-year-old child with G6PD deficiency on D4, D5 and D6 (from left to right) after the 4th daily dose of primaquine 15mg. At admission to the pediatric emergency hospital of Wad Medani, the child had Hb at 2 g/dL, corrected to 8 g/dL after blood transfusion (Dec 2018)
Primaquine dose dependent hemolysis


Haemolytic response following daily and weekly doses of primaquine in the same subject, a male volunteer with A- variant of G6PD deficiency

In a GSK-sponsored study of tafenoquine (TAF 110027), 4 heterozygous women were treated with 15 mg primaquine base for 14 days and showed a level of drop of Hb (2.5 g/dL) similar to that observed in all patients with G6PD deficiency. These women had G6PD activity levels ranging between 40% and 60% of normal.

Haemoglobin (orange, above) and reticulocyte (black, below) levels following daily primaquine for 14 days at 0.25 mg/kg/day among four women heterozygous for G6PD deficiency - Courtesy of GSK.
Patients were assigned to receive tafenoquine (single 300-mg dose), placebo, or primaquine (15 mg, administered once daily for 14 days) in addition to a 3-day course of chloroquine (total dose of 1500 mg).


Krintafel® (tafenoquine) prescribing information:
• Contraindication: G6PD deficiency or unknown G6PD status
• Patients were excluded from clinical trials of Krintafel if they had a G6PD enzyme activity level <70% of the site median value for G6PD normal activity

https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210795s000lbl.pdf
New point of care G6PD diagnostics

Point-of-care tests for G6PD deficiency

<table>
<thead>
<tr>
<th>Quantitative</th>
<th>Qualitative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COMMERCIALY AVAILABLE</strong></td>
<td><strong>IN DEVELOPMENT</strong></td>
</tr>
<tr>
<td>G6PD Biosensor AccessBio</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>STANDARD G6PD SD Biosensor</td>
<td></td>
</tr>
</tbody>
</table>

**Common features**

- Finger prick blood, result in < 10 mins
- 12 month shelf life
- Storage 25-30°C

**Quantitative read-out; analyzer, multi-steps**
- Adjustment for Hb and temperature
- Required for tafenoquine

**RDT-like format**
- Discriminate < and > 30% activity (ok for males); subjective read-out
- No adjustment for Hb and temperature
Brief History of G6PD Classification

- WHO scientific Group – 1967
- WHO working group 1985 - Published 1989 in Bulletin of WHO

Human Glucose-6-Phosphate Dehydrogenase Variants*
by Akira Yoneda,1 Ehret B. Boulter,3 & Arno G. Motulsky* 2

So many glucose-6-phosphate dehydrogenase (G6PD) variants have been described that it has become very difficult to determine whether or not a newly discovered variant is distinct from any other. This difficulty can be partially overcome by performing a number of physicochemical tests and comparing the results with those already reported for the known variants. The purpose of this communication is to provide an up-to-date table summarizing the currently available data on G6PD variants. For purposes of convenience, the variants described in the accompanying table are somewhat arbitrarily divided into five classes, in accordance with their activity in red cells and their associated clinical manifestations:

Class 1: Severe enzyme deficiency with chronic non-spherocytic haemolytic anaemia.
Class 2: Severe enzyme deficiency (<10% of normal).
Class 3: Moderate to mild enzyme deficiency (10-40% of normal).
Class 4: Very mild or no enzyme deficiency (40-100% of normal).
Class 5: Increased enzyme activity (more than twice normal).

The distinction between these classes is not always clear. For example, G6PD Mediterranean has been placed in class 1, but has been reported to be associated with non-spherocytic congenital haemolytic anaemia. Furthermore, some of the variants listed in class 1, because of the severe functional lesions they cause, actually have higher enzyme activities in vivo than some of the variants with "moderate to mild enzyme deficiency" (class 3). Within the classes, the variant enzymes are arranged in order of their electrophoretic mobility—i.e., the fastest one is first.

The variants of each class are also subdivided into four groups according to the degree of their characterization, as tabulated in the report of the WHO Scientific Group on the Standardization of Procedures for the Study of Glucose-6-Phosphate Dehydrogenase (1985).

Group I: Variants have been fully characterized and appear to be distinct.
Group II: Insufficient information is available to be reasonably certain that it is unique. These variants are shown with quotation marks around the name of the variant.
Group III: Variants have been described, but insufficient data have been given to warrant their inclusion in the table.
Group IV: Variants have been characterized, but seem to be identical to one of the variants listed in the table.

The data in the table are the raw values given in the reports; no critical judgement of their dependability or accuracy has been made. In general, values of the Michaelis constant (Km) for NADP, particularly those of deficient variants, may not be accurate. Therefore, differences of Km for NADP alone cannot be used as a critical factor in distinguishing variants.

In order to distinguish closely similar variants, parallel comparisons under the same conditions should be performed. Unfortunately, many blood banks are not equipped to perform these comparisons. To familiarize the reader with the problem, several comparisons are shown in Figure 1. For the purposes of this article, the term NADP refers to diaphorase, i.e., the oxidized form of NADP. The reduced form (NADPH) is the end product of G6PD in the process. NADPH is required for regeneration of G6PD by the enzyme glutathione reductase. It is also considered to be the most important factor in the regulation of G6PD in the body. In other tissues where NADP is already low, no additional enzyme activity can be measured. The lack of activity in the red cells is probably due to an enzyme deficiency.

* This work is supported by grants from the World Health Organization (International Reference Center for G6PD Variants), and by US Public Health Service grants GM 17115 and HD 21449 from the National Institutes of Health, the National Heart, Lung, and Blood Institute, and the National Institute of General Medical Sciences, and the Damon Runyon-Williams Memorial Fund. 1 Research Professor, Department of Medicine (Division of Medical Genetics), University of Washington, Seattle, Wash., USA. 2 Chairman, Division of Medicine, City of Hope National Medical Center, College of Medicine, Los Angeles, Calif., USA. 3 Professor of Medicine and Genetics, University of Washington, Seattle, Wash., USA.

Update/Le point

Glucose-6-phosphate dehydrogenase deficiency*
WHO Working Group

Glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency) is the commonest enzyme deficiency of humans and is a globally important cause of maternal jaundice, which can lead to haemolysis and death or sequelae such as cerebral palsy. It is also an important cause of morbidity in children and adults. The condition is caused by a deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD), which is largely by birth, education, and information, and maternal jaundice can be successfully treated by phototherapy, a cheap and easy treatment approach suitable for use in primary health care.

This update describes developments in the methodology for characterizing G6PD deficiency, the knowledge of the factors that cause haemolysis, the community strategy for prevention of haemolytic crises in newborn infants, and the implications of red cell prevaience at the DNA level.
Proposed classification schemes

**WHO Scientific Group 1966**

- List of variants; no formal classification
- Clear phenotypic separation of: *acute haemolytic anaemia* (AHA) versus *chronic haemolytic anaemia* (CNSHA)
- (-) indicates 25% or less activity; (+/-) indicates 25-65% activity; (+) indicates normal activity (65-150%); (++) indicates greater than 150% activity

**Yoshida, Beutler & Motulsky, Bulletin of WHO, 1971:**

List of variants in 5 classes:

- Activity <10% of normal, **severe** enzyme deficiency with **chronic non-spherocytic haemolytic anaemia** (CNSHA).
- Activity <10% of normal, **severe** enzyme deficiency
- Activity 10-60% of normal, **moderate to mild** enzyme deficiency, intermittent acute hemolysis
- **Very mild or no enzyme** deficiency (60-100% of normal)
- Increased enzyme activity (more than twice normal)

Cautionary statements: “for purposes of convenience, the variants described in the accompanying table are somewhat arbitrarily divided into five classes” and after “the distinction between these classes is not always clear”

**Since 1971 this has been referred to as the ‘WHO classification’**
WHO working group 1985

- Class I - associated with chronic non-spherocytic haemolytic anaemia (CNSHA)
- Class II - severely deficient: less than 10% residual activity
- Class III - moderately deficient: 10-60% residual activity
- Class IV - normal activity: 60-150%
- Class V - increased activity

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Current WHO G6PD classification

- Class I - associated with chronic non-spherocytic haemolytic anaemia (CNSHA)
- Class II - severely deficient: less than 10% residual activity
- Class III - moderately deficient: 10-60% residual activity
- Class IV - normal activity: 60-150%
- Class V - increased activity
Distribution of a sample of 20 polymorphic G6PD variants in relation to mean residual enzyme activity

(courtesy of Prof L. Luzzatto)
Distributions of enzyme activity among samples male subjects with 2 different variants of G6PD deficiency

Additional reasons to update the G6PD classification:

- Variable definitions of normal (> 70%, >80% residual activity)
- Only one reported case with > 150% activity
- Combine biochemical and molecular characterization

Courtesy of Prof. Lucio Luzzato
Propose to revise based on new classification and additional clinical manifestations
Serendipitous finding: ICD no capture of G6PD deficiency as genetic condition, only as it is associated with clinical manifestation (AHA)

**ICD-11: 3A10.00 – haemolytic anaemia due to G6PD deficiency**

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary erythrocyte enzyme deficiency that can manifest with severe neonatal jaundice which can lead to serious neurological consequences, or, most often, with acute hemolytic anemia following ingestion of certain foods (fava beans), common drugs (some antimalaria drugs, sulphamides, analgesics), or in the course of an infection, in otherwise asymptomatic individuals.

- Neonatal screening not captured
- Point of care testing options may expand
- Process for revising ICD is based on expert proposal
- Next revision February 2020
Many thanks for your kind attention