Glucose 6 phosphate dehydrogenase deficiency

Prof. Renzo Galanello
Pediatric Clinic 2° University of Cagliari
Ospedale Regionale Microcitemie-ASL8
Role of G6PD in glucose RBC metabolism

- 1% use in normal conditions.
- More than 10% in oxidative stress.

Glucose → G6P → 6PG

- G6PD
- Pentose-phosphate pathway
- NADPH

- Glycolysis
- ATP

- Metabolic reactions
- Ribose-5-P for nt synthesis

Energy
LOCALIZZAZIONE DEL GENE G6PD SUL CROMOSOMA X E SUA STRUTTURA FISICA

Xq27

R/G CV
G6PD
FVIIIIC

Xq28

1       2         3

Megabasi

5’

3’

13 Kb

FVIIIIC G6PD G G G R

1  2  3
G6PD PROTEIN

Amino Acids, number
515 (512)

Molecular Weight
59kD

Subunits per molecule of active enzyme
2 or 4

Molecules of tightly bound NADP per subunit
1
HEREDITARY TRANSMISSION OF G6PD DEFICIENCY

G6PD DEFICIENCY
## Genetic Polymorphism of G6PD

<table>
<thead>
<tr>
<th>CLASS</th>
<th>Clinical Expression</th>
<th>Residual Activity (% of normal)</th>
<th>Altered Electrophor. Mobility %</th>
<th>No. of Variants</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Severe (CNSHA)</td>
<td>&lt; 5</td>
<td>63</td>
<td>94</td>
<td>Harilaou, Telti</td>
</tr>
<tr>
<td>II</td>
<td>Moderate</td>
<td>&lt; 10</td>
<td>64</td>
<td>114</td>
<td>Mediterranean</td>
</tr>
<tr>
<td>III</td>
<td>Mild</td>
<td>10-60</td>
<td>82</td>
<td>110</td>
<td>A^-</td>
</tr>
<tr>
<td>IV</td>
<td>None</td>
<td>100</td>
<td>95</td>
<td>52</td>
<td>A^+, B</td>
</tr>
<tr>
<td>V</td>
<td>None</td>
<td>&gt; 100</td>
<td>100</td>
<td>2</td>
<td>Hektoen, Verona</td>
</tr>
</tbody>
</table>
## TYPES OF MUTATIONS IN G6PD GENE

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>Substitution</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Missense</strong></td>
<td>1 nucleotide</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>2 nucleotides*</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>3 nucleotides**</td>
<td>1</td>
</tr>
<tr>
<td><strong>Deletion</strong></td>
<td>1 codon</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2 codon</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8 codon</td>
<td>1</td>
</tr>
<tr>
<td><strong>Non-sense</strong></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Splicing</strong></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

* Double aminoacid substitution in the enzyme
** Triple aminoacid substitution in the enzyme (es. G6PD Vancouver)
Although the activity of the normal enzyme declines as red cells age, even the oldest cells have a sufficient level of activity to provide protection against oxidative damage and hemolysis.

By contrast, very few G6PD Mediterranean red cells have sufficient enzyme activity to prevent oxidative damage, whereas a substantial fraction of young G6PD A− red cells are able to provide protection.

Figure 13.13
Decline of erythrocyte G6PD activity with cell age for the three most commonly encountered forms of the enzyme.
World map distribution of G6PD deficiency

WHO Working Group., 1989
Distribution of G6PD, Fava Beans, and Malaria
Location of nucleotide substitutions causing glucose-6-phosphate dehydrogenase (G6PD) deficiency

Mehta et al, 2000
Distribution of the males G6PD deficient in Sardinia
(11763 males from 1998 to 2007)

(5B Project)
<table>
<thead>
<tr>
<th>Variant</th>
<th>Base change</th>
<th>Ex</th>
<th>Aminoacid change</th>
<th>Electroph. Mobility (% of GdB)</th>
<th>Activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediterranean</td>
<td>563 C→T</td>
<td>VI</td>
<td>188 Ser→Phe</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td>Union</td>
<td>1360 C→T</td>
<td>XI</td>
<td>454 Arg→Cys</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td>S.Antioco</td>
<td>1342 A→G</td>
<td>XI</td>
<td>448 Ser→Gly</td>
<td>100</td>
<td>22</td>
</tr>
<tr>
<td>Seattle</td>
<td>844 G→C</td>
<td>VIII</td>
<td>282Asp→His</td>
<td>100</td>
<td>36</td>
</tr>
<tr>
<td>Sinnai</td>
<td>34 G→T</td>
<td>II</td>
<td>12 Val→Leu</td>
<td>100</td>
<td>67</td>
</tr>
</tbody>
</table>
PREVALENT ERYTHROID MANIFESTATIONS OF G6PD DEFICIENCY

- RBC unable to renew G6PD supply
- NADPH production in RBC only from Pentose Phosphate Pathway (HM shunt)
Clinical manifestations of G6PD deficiency

- Drug induced hemolytic anemia
- Infection induced hemolytic anemia
- Favism
- Neonatal jaundice
- Congenital non-spherocytic hemolytic anemia
AGENTS ASSOCIATED WITH HEMOLYSIS FROM G6PD DEFICIENCY

- **drugs** (see list)
- **fava beans**
- **chemicals:** benzene, naphtalene
- **Infections:** hepatitis, infectious mononucleosis, respiratory infections, sepsis
- **diabetes:** acidosis, hypoglicemia
MECHANISM OF HEMOLYSIS

• Inability to maintain adequate levels of GSH, leads to disulfide aggregates attaching to RBC membrane

• Neutralization of toxic free O\textsuperscript{-} radicals is impaired, and H\textsubscript{2}O\textsubscript{2} accumulates.

• Hb oxidation and molecular rupture lead to Heinz body formation causing membrane alterations
Broad beans characteristics

- neolithic era (3000 B.C.)
- moderate climate
- fast growth in winter/spring
- irrigation not necessary
- content:
  - proteins 22-26%
  - starch 43-45%
  - lipids 2.5%
Favism: symptoms

• Sudden rise of body temperature and yellow coloring of skin and mucous membrane.

➢ Dark yellow-orange urine.

• Pallor, fatigue, general deterioration of physical conditions.

• Heavy, fast breathing.

• Weak, rapid pulse.
FAVISM

CHARACTERISTICS

- Med, Canton and A- (rare)
- Most common: 2 – 6 year
- Amount and type of fava beans (young, raw higher risk)
- Similar to drug associated hemolytic crisis
- Variable individual susceptibility: variable absorption of vicin other factors
- Erratic crisis (in adults 25 % of the cases)
- With breast feeding
Vicine, convicine and the respective aglycones
Urine changes over time in a patient with favism
Symptoms from pollen in G6PD deficient subjects

Headache, nausea, diarrhea:
proximity to fava beans in flower (several reports)

Mild jaundice, dark urine:
contact with pod, fava beans while cooking

Headache, malaise, jaundice, vomiting, pallor, dark urine, fatigue:
proximity to flowering fava beans (one report Brodribb, 1966)
Hemolytic anemia from drugs or infections in G6PD deficient subjects

• Clinical manifestations are similar to favism
Neonatal jaundice in g6pd deficient newborns

- Onset: 2 to 4 days
- Bilirubin higher than 150 micromol/L
- Variable severity: mild to kernicterus
- Very moderate anemia
- Pathophysiology: impaired liver function, hemolysis (minor contribute)
- Influence from environmental (maternal exposure to oxidants, herbal remedies, camphor balls) and genetic (gilbert syndrome genotype, Sephardic Jews) factors
G6PD ASSOCIATED NEONATAL JAUNDICE

management:

- prevention
- phototherapy
- phenobarbital
- exchange-transfusion (bilirubin > 300 μm/L)
CNSHA: WHO Class I deficiency

- Chronic anemia with increased retics
- Occur in males
- Probable severe NNJ
- Variability in clinical expression
- Risk of acute hemolysis with oxidant conditions
- Enzyme mutation is sporadic, so that each family usually has a different enzyme defect
- Supportive therapy, avoid oxidants, splenectomy
G6PD deficiency management

- Not available
- Not needed in normal conditions except CNSHA
- Supportive treatment for acute hemolysis
- Remove oxidants in hemolytic crisis
- Prevention: avoid oxidants, do not eat fava beans
DIAGNOSIS OF G6PD DEFICIENCY

**BIOCHEMICAL**

- **Screening assays**
  - Fluorescent spot test
  - Ascorbate cyamide test
  - MTT (tetrazolium) staining test

- **Definitive assays**
  - Spectrophotometry
  - Differential pH-metry
  - MetHb reduction methods
  - Dye decolorization

**GENETIC**

- DNA PCR based methods