

G6PD Deficiency: From Molecular Mechanisms to Clinical Implications

Bridging Basic Science, Clinical Practice,
Global Health

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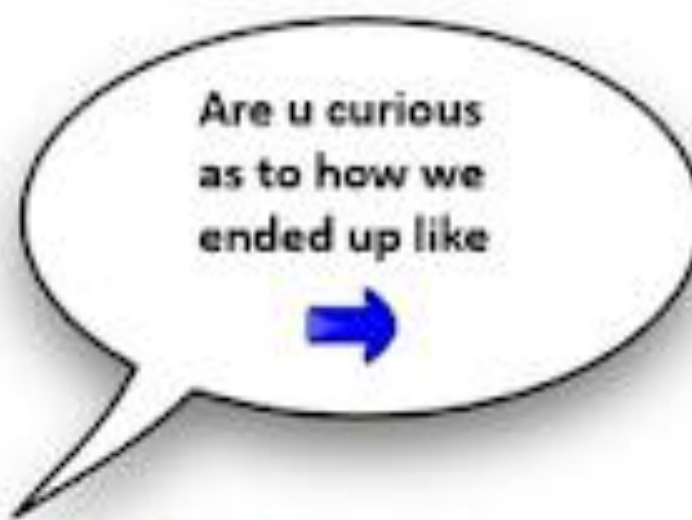
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Outline

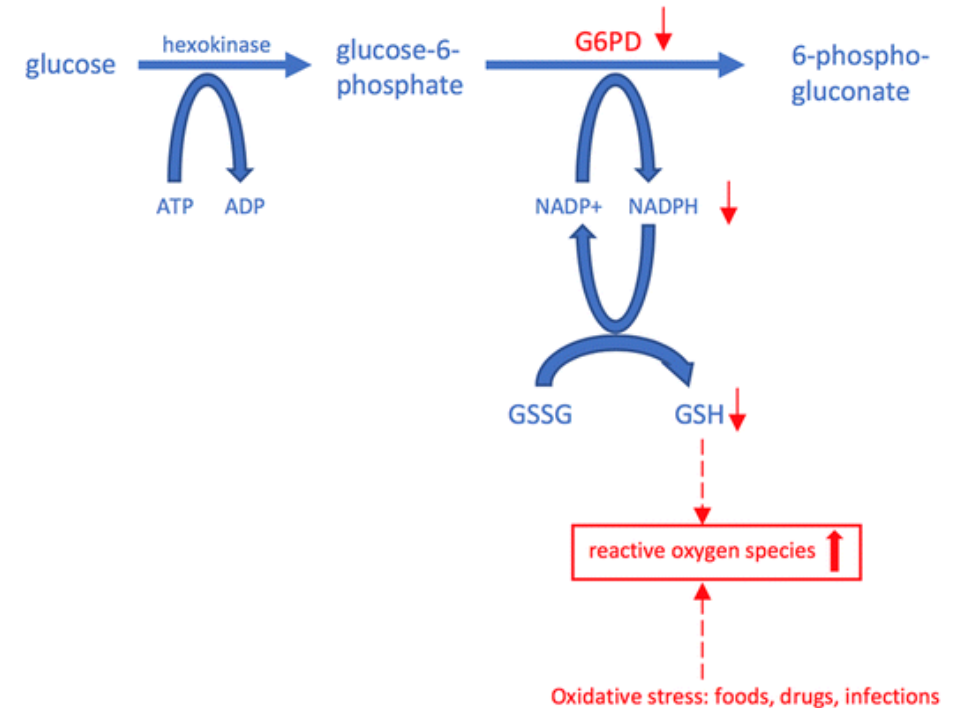
- Introduction to G6PD and Its Biological Role
- Genetics and Epidemiology
- Pathophysiology and Variants
- Clinical Manifestations and Triggers
- Diagnostic Approaches
- Management and Pharmacogenomics
- Pitfalls
- Global Health and Research Frontiers



- “G6PD deficiency is the most common enzymopathy worldwide, affecting ~4% of the global population.”
- Critical enzyme in the pentose phosphate pathway (PPP).

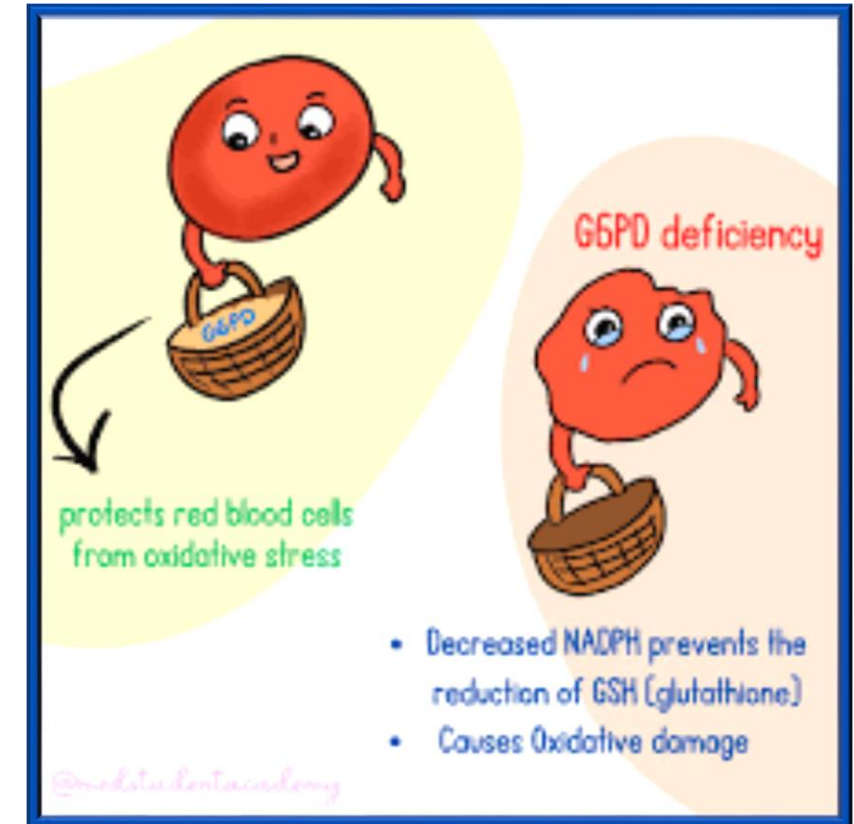
Introduction to G6PD

- NADPH:
- NADPH, a crucial reducing agent, is primarily produced in the pentose phosphate pathway, where glucose-6-phosphate dehydrogenase (G6PD) catalyzes the first step.
- G6PD converts glucose-6-phosphate into 6-phosphogluconolactone, simultaneously reducing NADP^+ to NADPH.
- NADPH, maintain reduced glutathione (GSH), protecting RBCs from oxidative damage



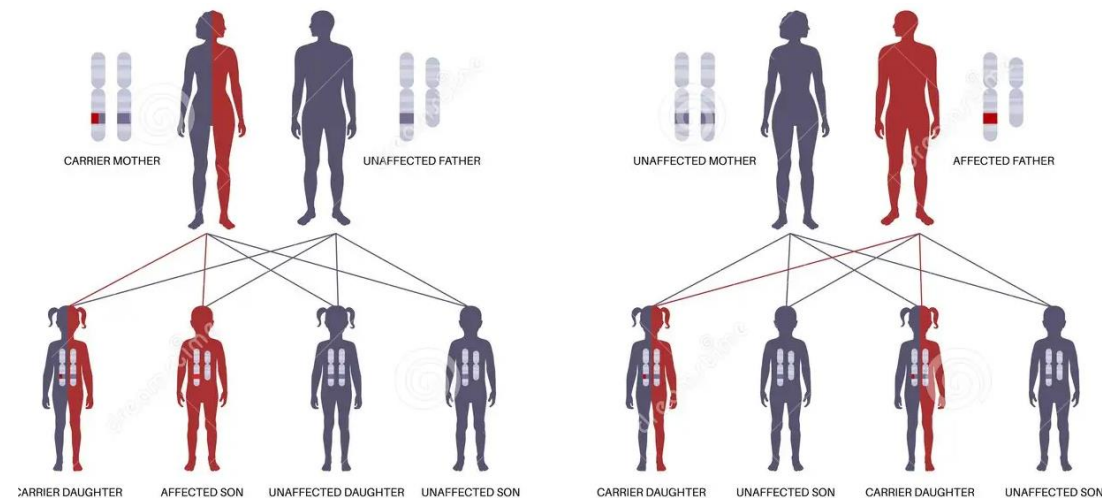
Introduction to G6PD

- Role in RBCs: Sole source of NADPH via the pentose phosphate pathway.
- Why RBCs? Lack mitochondria → rely solely on PPP for redox balance.



Genetics

- Inheritance: X-linked recessive (Xq28 locus)
- Males: Hemizygous → always affected.
- Females: Homozygous (rare) or heterozygous (variable expression due to Lyonization).

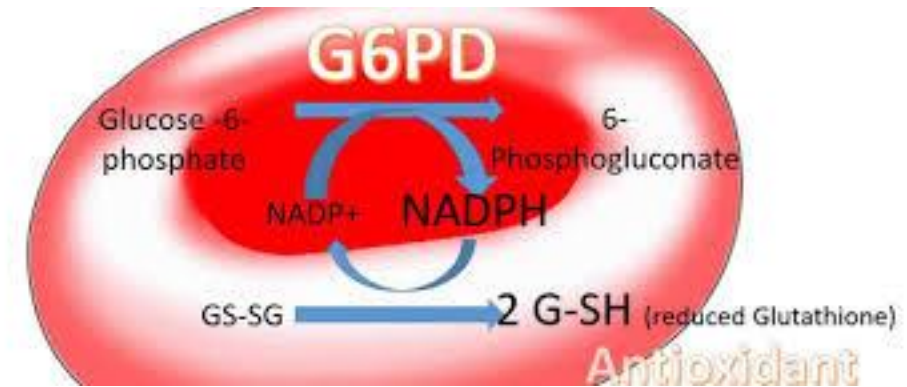


Genetics

- Common Variants:
- Class I (e.g., Canton): <10% activity, Severe deficiency, chronic hemolysis.
- Class II (e.g., Mediterranean): <10% activity, episodic hemolysis.
- Class III (e.g., A⁻): 10–60% activity, milder symptoms.

Recent Advances in G6PD Deficiency: Classification

- Revised WHO Classification (2023)
- New Class B: Combines former Class II (<10% activity) and Class III (10–60% activity) variants due to overlapping clinical risks (e.g., neonatal hyperbilirubinemia, drug-induced hemolysis).
- Threshold Update: Variants with enzyme activity <45% now categorized as clinically significant, emphasizing risks even in "moderate" deficiency.
- Implication: Neonates with Class B variants (e.g., G6PD A-) require vigilant monitoring for jaundice and hemolysis triggers.



Revised WHO Classification (2023)

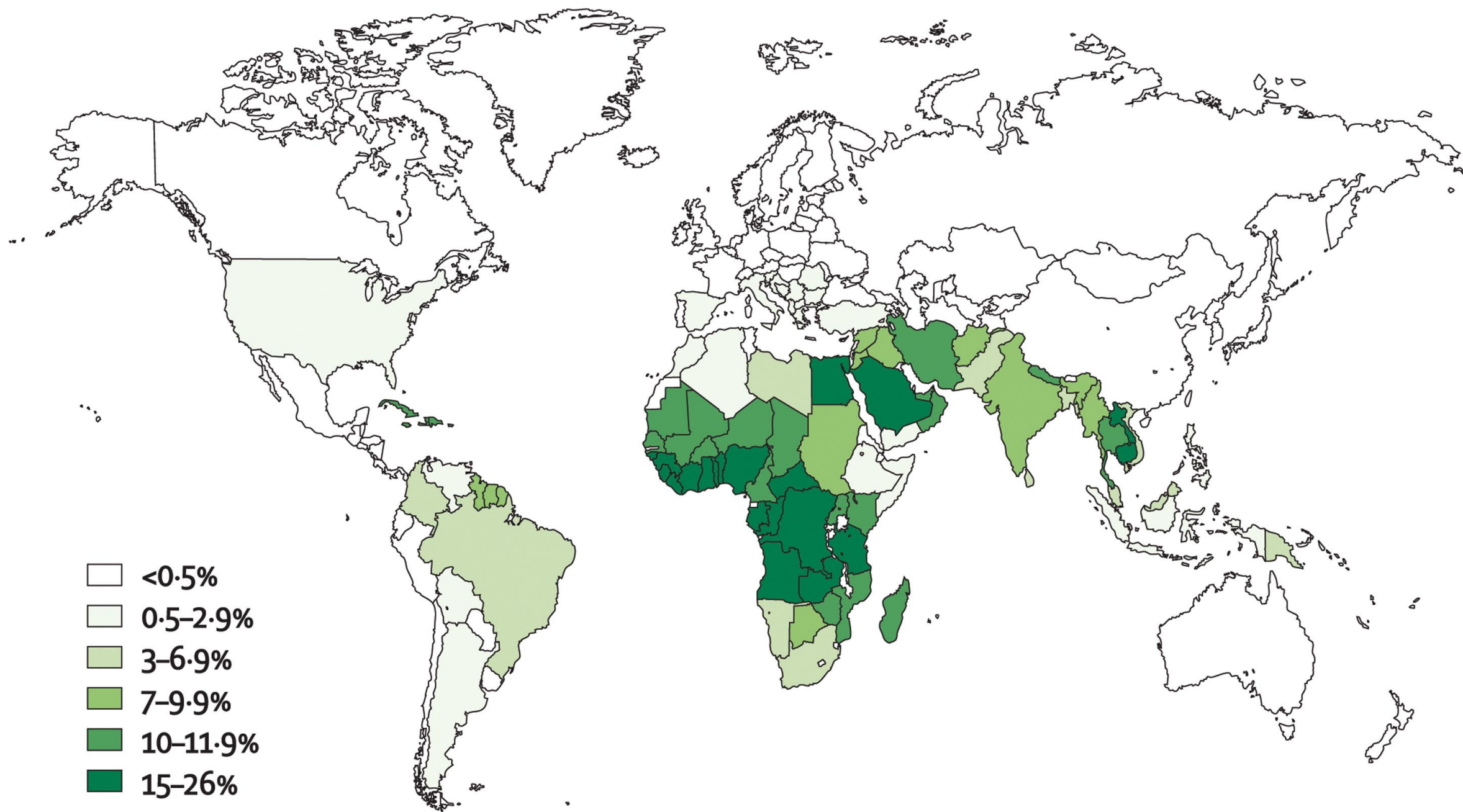
Comparison: Legacy vs. Revised WHO Classification

2023 WHO Class	Median Activity	Clinical Risk	Legacy WHO Class
A	<20%	Chronic hemolysis (CNSHA)	I
B	<45%	Acute hemolysis	II + III
C	60–150%	No risk	IV
U	Any	Uncertain significance	—

Epidemiology

- Over 200 G6PD variants have been described
- 400 million affected worldwide
- High in Africa, Mediterranean, Middle East, Southeast Asia
- Malaria Hypothesis:
- G6PD-deficient RBCs resist Plasmodium invasion.





Pathophysiology

- Oxidative stress → NADPH depletion → Heinz bodies → RBC lysis
- Heinz body : collections of denatured globin chains often attached to the RBC membrane





Pathophysiology Triggers

Drugs to Avoid in G6PD Deficiency

- Primaquine: Contraindicated; high risk of hemolysis.
- Tafenoquine: Avoid (similar to primaquine).
- Chloroquine/Hydroxychloroquine: Generally safe for acute malaria treatment but use cautiously.

Drugs to Avoid in G6PD Deficiency

Medicines and other substances likely to be UNSAFE in moderate to severe G6PD deficiency*	
Medications	
Chlorpropamide	
Dabrafenib	
Dapsone (diaminodiphenyl sulfone)	
Fluoroquinolones (ciprofloxacin, moxifloxacin, norfloxacin, ofloxacin) [¶]	
Methylene blue (methylthioninium chloride) ^Δ	
Nalidixic acid [◇]	
Nitrofurantoin, nifuratel, and nitrofurazone (nitrofur) [◇]	
Phenazopyridine (pyridium)	
Primaquine and tafenoquine	
Rasburicase and pegloticase	
Sulfonylureas (eg, glipizide, glyburide [glibenclamide])	

Drugs to Avoid in G6PD Deficiency

Medicines that are **PROBABLY SAFE** given in usual therapeutic doses in G6PD deficiency*; NOTE: some of these were previously considered unsafe; safety in Class I variants is generally not known

Acetaminophen (Tylenol, Paracetamol)	Chloroquine and hydroxychloroquine
Aminophenazone, dipyrrone, and metamizole (NSAIDs) [†]	Colchicine
Antazoline (antihistamine)	Clotrimazole
Antipyrine (phenazone)	Diphenhydramine (Benadryl)
Ascorbic acid (vitamin C)	Isoniazid
Aspirin (acetylsalicylic acid)	Levodopa (L-Dopa) and levodopa-carbidopa
Benzhexol (Artane)	Para-aminosalicylic acid
Chloramphenicol	Para-aminobenzoic acid (PABA)
	Phenylbutazone
	Phenytoin
	Probenecid (Benemid)
	Procainamide (Pronestyl)
	Pyrimethamine (Daraprim)
	Quinine



- Strongly associated with hemolytic episodes.
- The risk is not dose dependent and even low or short-term doses can cause sudden hemolysis
- Should be avoided unless absolutely necessary and under specialist supervision
- **Clinical judgment** should be used in deciding if it is safe for a specific individual; if it was used previously and found to be safe (eg., before the diagnosis was made) it may be reasonable to treat it as safe for that individual.



Antipyretic Analgesics



- Is generally safe when used in recommended dose



In typical doses is not likely to trigger hemolysis



ASA & G6PD deficiency

- 75 – 100 mg daily is often considered safe but only under medical supervision
- Over 500 mg may trigger hemolysis



- Low to moderate dose:
- Under 500 mg/day are usually safe
- High dose:
- Over 1gram/day IV or orally may pose a risk



- Vitamin K1 (phylloquinone) is the standard and safe form given to newborns is safe.
- Vitamin K2 (menaquinone) in fermented foods and supplements is safe
- Vitamin K3 (menadione) synthetic form is not safe
- Vitamin K is generally safe for newborns with G6PD deficiency but only the correct form and dose should be used



Pathophysiology

- Triggers:
- Infections.
- Viral/bacterial.



Pathophysiology

- Dietary: Fava beans (favism)
- Divicine in fava beans induces oxidative damage.



G6PD Deficiency: Food Safety Guide

- Foods to Avoid (High Risk of Hemolysis)
 - 1. Fava beans (broad beans) - Most well-known trigger; can cause "favism"
 - 2. Bitter melon (in large amounts) - May have pro-oxidant effects
 - 3. Artificial food colorings/preservatives - Especially sulfites and benzoates



G6PD Deficiency: Food Safety Guide

- Substances in Traditional or Herbal Products to Avoid
 - - Henna (especially in children - can be absorbed through skin)
 - - Mothballs (naphthalene) - Severe hemolysis risk if inhaled or ingested
 - - Camphor-containing products

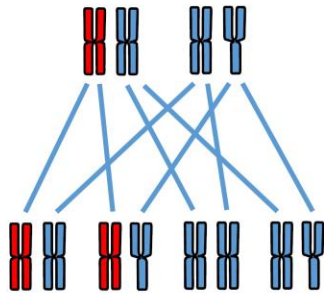
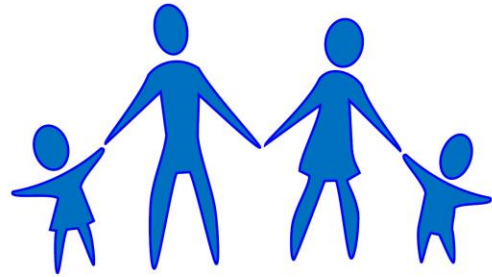


G6PD Deficiency: Food Safety Guide

- Myths and Clarifications
 - - Soy products: Usually safe, unless processed with unsafe additives
 - - Legumes other than fava beans: Generally safe (e.g., lentils, peas, chickpeas)
 - - Citrus fruits: Safe - no link to hemolysis
 - - Garlic/onion: Safe - not a known risk despite sulfur content

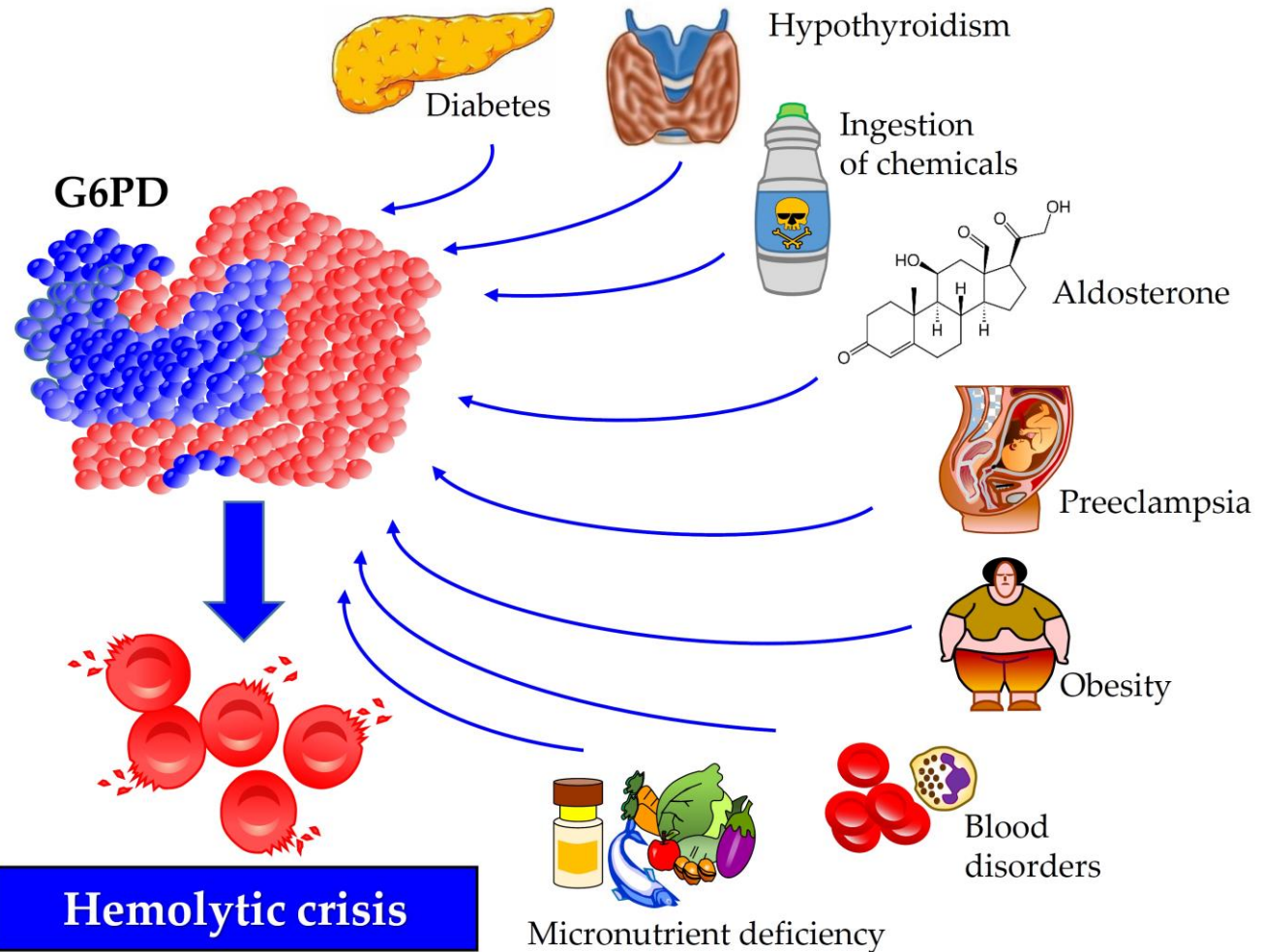


Inherited G6PD deficiency permanent



X-linked G6PD inheritance

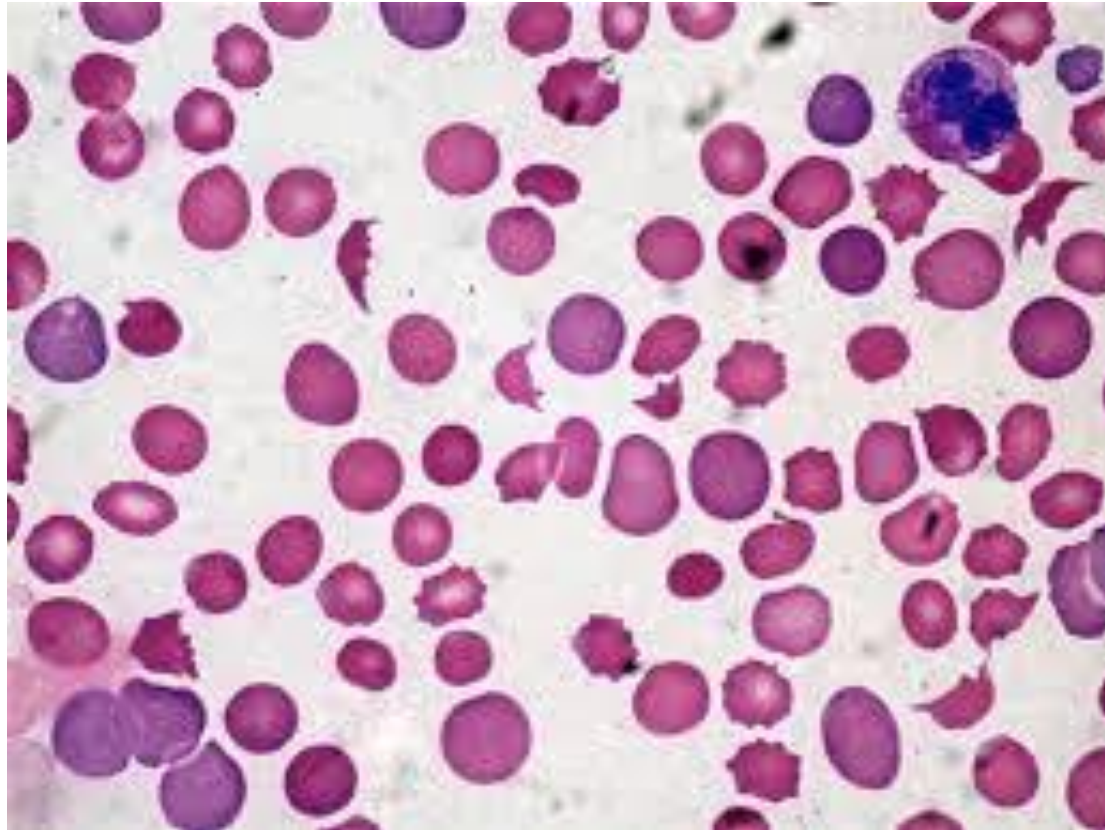
Acquired G6PD deficiency transient



Clinical Condition	In Vitro Studies	In Vivo Studies	Studies in Humans
Blood disorders	–	–	Somatic mutation in bone marrow progenitor cells [18]; hematopoietic stem cell transplantation [20]; transfusion from deficient donors [21,22]
Ingestion of chemicals	–	–	Sodium chlorite [17] Herbal supplements [23]
Endocrine disorders	Excess of mineralocorticoids [24] Hypothyroidism [25]	Hypothyroid state by using drugs [26,27,28], Thyroidectomy [29] Polycystic ovary [30] Streptozotocin-induced diabetes [38]	Excess of mineralocorticoids [31] Congenital hypothyroidism [32] Diabetes [33,34,35] Ketosis-prone diabetes [36,37] Rheumatoid arthritis associated with metabolic syndrome [19]
Preeclampsia	–	–	Impaired redox status [39,40]
Micronutrient deficiency	–	Rat duodenal mucosa [41]; vitamin D deficiency [42]	Zinc deficiency [43]

Clinical Manifestations

- Acute Hemolytic Anemia: Dark urine, fatigue, jaundice



Neonatal Jaundice



Clinical Manifestations

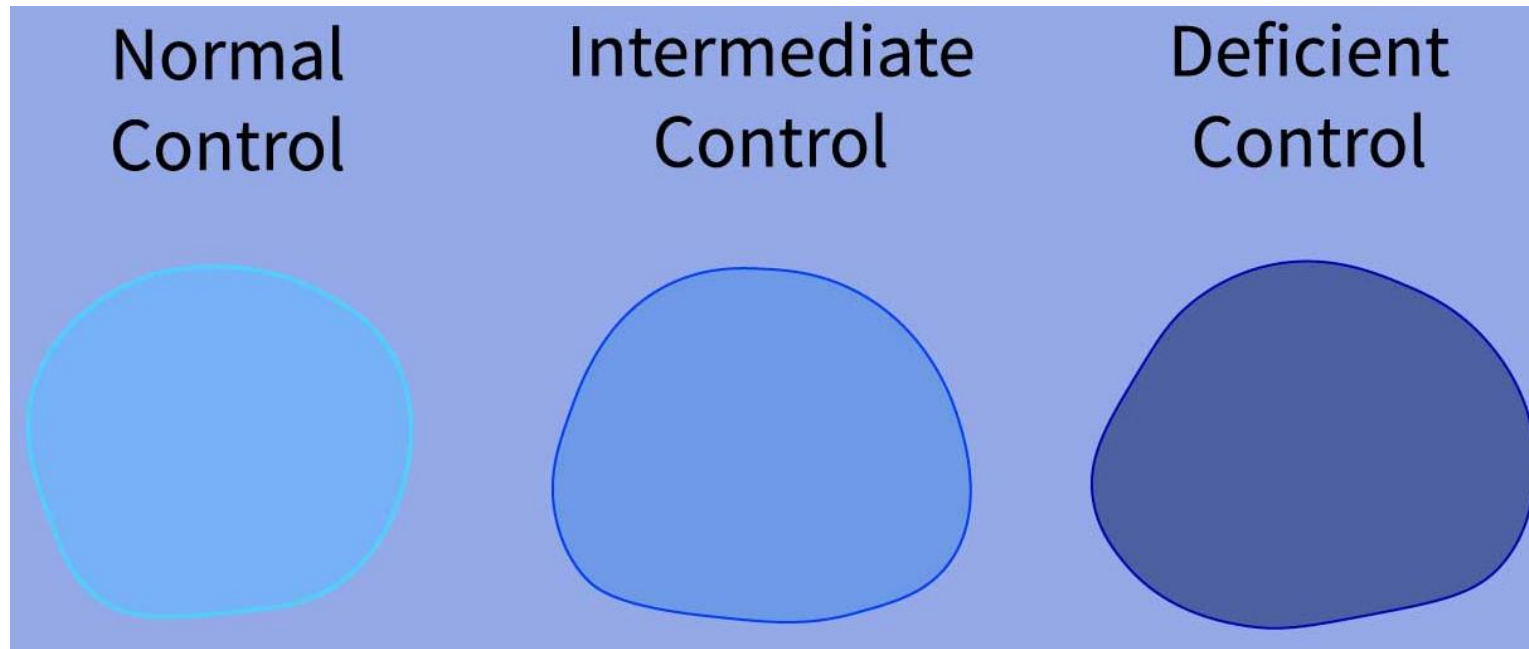
- Chronic Nonspherocytic Hemolytic Anemia (Class I variants)

Clinical Manifestations

- Neutrophil dysfunction
- G6PD is used by other cells besides RBCs to reduce oxidant injury.
- Rarely, individuals with severe G6PD deficiency (eg, <20 percent activity at baseline) may have neutrophil dysfunction due to an impaired respiratory burst, with impaired bactericidal activity and recurrent infections with catalase-positive organisms.

Diagnosis

- Screening Tests:
- Fluorescent spot test (qualitative NADPH detection).



Diagnosis

- Confirmatory: Quantitative enzyme assay
- Genetic Testing: PCR for common variants



Management

- **Avoid Triggers**





- Acute Hemolysis: Hydration, transfusion

Why Hydration Matters

- Kidney Protection:
- Hemolysis releases free hemoglobin into the bloodstream, which can clog renal tubules and cause acute tubular necrosis (ATN).
- Adequate hydration maintains renal blood flow, dilutes hemoglobin, and promotes its excretion, reducing kidney damage risk.
- Electrolyte Balance:
- Hemolysis releases potassium from ruptured red cells, increasing the risk of hyperkalemia (high potassium). Hydration helps stabilize electrolytes.

Hydration Strategies

- Oral Hydration (if mild/moderate hemolysis):
 - Encourage frequent sips of water or oral rehydration solutions (e.g., WHO-ORS).
 - Avoid sugary drinks or caffeine, which can worsen dehydration.
- Intravenous (IV) Fluids (if severe hemolysis or vomiting):
 - Isotonic saline (0.9% NaCl) is often used to maintain intravascular volume and renal perfusion.
 - Balanced crystalloids (e.g., Ringer's lactate) may also be used, depending on electrolyte levels.

Sodium bicarbonate

- Should be used only in severe hemolysis with concern for kidney damage
- Not routinely in mild or moderate hemolysis without renal involvement



Monitoring:

A hand wearing a blue nitrile glove holds a test tube with a barcode label. The test tube contains a red liquid. In the background, a rack holds several other test tubes with different colored caps (yellow, red, blue, green). The background is blurred, showing a laboratory setting.

- Track urine output (goal: $>0.5\text{--}1$ mL/kg/hour). Dark urine (cola-colored) suggests ongoing hemolysis.
- Check creatinine, BUN, and potassium levels to assess kidney function and electrolyte status.

Transfusion



- Hemoglobin lower than 7 g/dl or symptomatic anemia regardless of Hb level
- Hemodynamic instability
- Ongoing hemolysis with inadequate compensatory erythropoiesis



- Neonatal Jaundice: Phototherapy/exchange transfusion

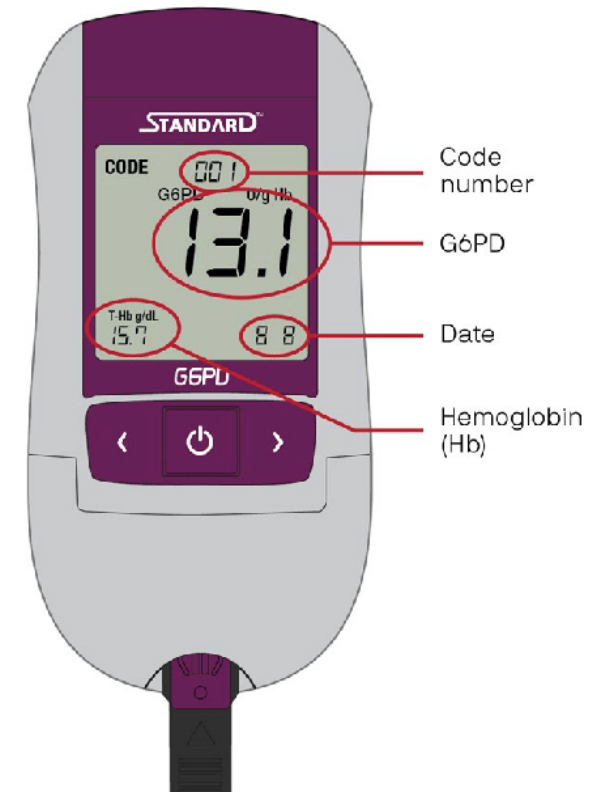
Global Health Impact

- Challenges in Malaria Treatment: Balancing radical cure (vivax/ovale) vs. hemolysis
- Screening Programs: Neonatal vs. population-based (cost-effectiveness)



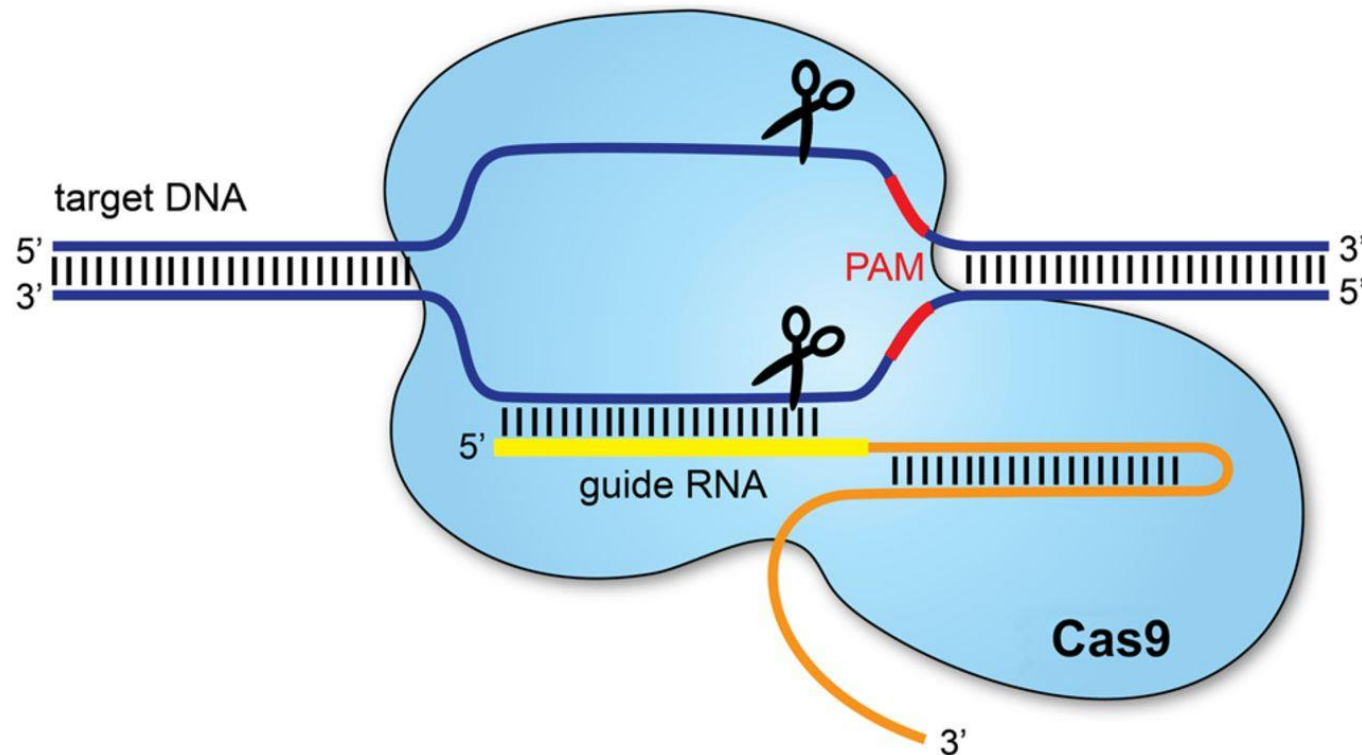
Global Health Impact

- Point-of-Care Testing: Biosensors for G6PD activity in resource-limited settings
- Biosensors (e.g., STANDARD G6PD test) enable safe primaquine use in endemic areas



Research Frontiers

- Gene Therapy: CRISPR/Cas9 approaches (preclinical studies)
- CRISPR editing in hematopoietic stem cells (preclinical success in mice).



Research Frontiers

- Drug Development: Tafenoquine (single-dose antimalarial with safer profile)



Discussion Points



Heterozygous Females: To Screen or Not to Screen?

- Challenge:
- Lyonization (X-inactivation) → mosaicism → variable enzyme activity (10–90%).
- Some heterozygous females experience hemolysis, while others are asymptomatic.
- Clinical dilemma: Should they avoid triggers (e.g., primaquine) even if asymptomatic?
- “Should all heterozygous females undergo quantitative enzyme testing?”
- “How do we balance over-caution vs. under-treatment in this population?”
- Yes, especially in settings where G6PD deficiency is common or if there is a clinical history suggesting hemolysis risk

Universal Screening in Malaria-Endemic Zones: Cost vs. Benefit

- Prevents hemolysis in 1/20 males (Africa) and enables safe primaquine use for *P. vivax*.
- WHO recommends pre-primaquine testing (2023 guidelines).
- Cost of point-of-care tests (e.g., STANDARD G6PD \approx \$5/test) vs. limited health budgets.

Ethical Dilemmas in Malaria Treatment

- Conflict: Radical cure (primaquine/tafenoquine) vs. hemolysis risk.
- Scenario 1:
- P. vivax in a G6PD-deficient patient: Withhold primaquine → risk relapse → chronic morbidity.
- Scenario 2:
- Use primaquine cautiously → hemolysis → renal failure in resource-limited settings.

Emerging Therapies: Hope or Hype?

- Gene Therapy:
- Preclinical success in mice (CRISPR-Cas9 editing of hematopoietic stem cells).
- Ethical concern: Is germline editing justified for a non-fatal condition?
- Tafenoquine:
- Single-dose antimalarial but still unsafe in Class I/II deficiency.
- Is it equitable if 20% of the population in endemic zones cannot use it?

Policy Challenges: Neonatal vs. Prenatal Screening

- Neonatal Screening:
 - Prevents kernicterus.
 - Misses pre-pregnancy counseling opportunities.
- Prenatal Screening:
 - Empowers carrier mothers (X-linked counseling).
 - Stigma and abortion debates in conservative regions.

HbA1c Pitfalls in G6PD Deficiency: A Hidden Diagnostic Challenge

- G6PD-deficient RBCs lyse prematurely → reduced exposure to glucose → falsely low HbA1c (underestimates true glycemia).
- ↑ RBC turnover → ↓ time for hemoglobin glycation (HbA1c reflects average glucose over ~120 days).

HbA1c Pitfalls in G6PD Deficiency: A Hidden Diagnostic Challenge

- HbA1c may be 0.5–1.5% lower than actual mean glucose (Cohen et al., Diabetes Care, 2018).
- Risks:
- Undertreatment of diabetes → hyperglycemia complications (retinopathy, nephropathy).

HbA1c Pitfalls in G6PD Deficiency: A Hidden Diagnostic Challenge

- Alternatives to HbA1c
- Fructosamine:
 - Reflects 2–3 weeks of glycemia (albumin-bound glucose; less affected by hemolysis).
- Continuous Glucose Monitoring (CGM):
 - Provides real-time glucose trends.
- Glycated Albumin:
 - Useful in chronic hemolysis.

HbA1c Pitfalls in G6PD Deficiency: A Hidden Diagnostic Challenge

- Recommendations
- Screen for G6PD deficiency in diabetic patients with:
 - Unexplained low HbA1c + elevated glucose.
 - Ethnicity from high-prevalence regions (Africa, Mediterranean).
- Use HbA1c cautiously in G6PD deficiency; prioritize Fructosamine/CGM.

G6PD Deficiency in Neonates: Pitfalls in Diagnosis and Management of Hemolysis

- False-Negative G6PD Testing During Acute Hemolysis:
 - Reticulocytes have \uparrow G6PD activity \rightarrow mask deficiency in acute phase.
 - Solution: Repeat testing after 3 months (post-reticulocytosis).
-
- “Timing is everything—test too early, miss the truth.”



G6PD Deficiency in Neonates: Pitfalls in Diagnosis and Management of Hemolysis

- Management Errors
- Inadequate Phototherapy:
 - Delayed treatment due to underestimating bilirubin rise (non-linear progression).
 - Unsafe Transfusions:
 - Using blood from G6PD-deficient donors → risk of recurrent hemolysis.
- Overlooking Triggers:
- Maternal exposure to oxidative drugs (e.g., sulfa antibiotics) via breast milk.



Blood donation

- As a general rule, donated blood is not screened for G6PD deficiency, and individuals with G6PD deficiency can donate blood as long as they are otherwise able to donate and do not have anemia.
- This is because the typical lifespan of transfused G6PD-deficient RBCs is thought to be relatively normal, and it is unlikely for a patient to be transfused with multiple units of G6PD-deficient blood and have clinically significant hemolysis, even in areas of high prevalence.



- Cardiovascular Link: Emerging evidence associates G6PD deficiency with 71% higher atherosclerotic CVD risk (oxidative stress-driven endothelial dysfunction).

A hand is pointing at a circular graphic that contains the word "Updating". The background of the slide is a blurred image of a person in a white lab coat.

Updating

- Can we predict hemolysis severity using biomarkers beyond enzyme activity (e.g., oxidative stress markers)?



OPEN ENDED QUESTIONS

- How does G6PD deficiency interact with non-hematologic conditions (e.g., CVD, neurodegeneration)?
- Can AI models optimize trigger avoidance (e.g., drug-G6PD interaction databases)?

WHAT

HOW

WHERE

WHEN

WHY

WHO

Take Home Messages



"G6PD deficiency underscores the critical role of NADPH in protecting cells from oxidative damage; understanding this link highlights the importance of avoiding oxidative triggers to prevent hemolytic anemia and manage this common enzyme disorder."