Glucose 6-phosphate dehydrogenase deficiency: from genotype to phenotype

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Glucose 6-phosphate dehydrogenase (G6PD) is a housekeeping enzyme critical in the redox metabolism of all aerobic cells. G6PD deficiency has been a prototype of hemolytic anemias due to enzymopathy, i.e. to a primary abnormality of a red cell enzyme. G6PD deficiency is also a prime example of a hemolytic anemia due to an interaction between an intracorpuscular cause and an extracorpuscular cause, because in the majority of cases hemolysis is triggered by an exogenous agent.

Epidemiology

A distinction must be made between (i) the prevalence of G6PD deficiency as a genetic abnormality and (ii) the incidence of hemolytic anemia associated with G6PD deficiency. The genetic abnormality is distributed worldwide (Figure 1): a conservative estimate is that at least 400 million people carry a G6PD deficiency gene. Areas of high prevalence are Africa, Southern Europe, the Middle East, South-East Asia and Oceania. In the Americas and in parts of Northern Europe G6PD deficiency is also quite prevalent as a result of migrations in relatively recent historical times. Although accurate quantitative data are lacking, fava beans are probably still today the commonest trigger of hemolysis in G6PD-deficient subjects: therefore the incidence of this clinical manifestation can be identified with the epidemiology of favism. Fava beans are grown world-wide; they are a significant component of the diet particularly in the Middle East, in Iran and in Southern Europe.

Clinical manifestations

In view of the large number of people who have G6PD deficiency, it is important to note first of all that the vast majority remain clinically asymptomatic throughout their lifetime.

Neonatal jaundice

Although this is not always recognized, for reasons that are incompletely understood, G6PD deficiency is more likely to manifest during the neonatal period: indeed, the risk of developing neonatal jaundice is much greater in G6PD-deficient neonates than in G6PD-normal ones. The strength of the association between G6PD deficiency and neonatal jaundice appears to vary in different populations. The clinical picture of neonatal jaundice related to G6PD deficiency differs from the classical. Rhesus-related neonatal jaundice in two main respects: (i) it is very rarely present at birth, and the peak incidence of clinical onset is between day 2 and day 3; (ii) there is more jaundice than anemia, and the anemia is very rarely severe: in fact, it overlaps with physiological jaundice. Nevertheless, at the other end of the spectrum, neonatal jaundice can be very severe in G6PD-deficient babies, especially in association with prematurity, infection, and/or environmental factors (such as naphthalene-camphor balls, used in babies' bedding and clothing), and it can cause kernicterus. Unfortunately inadequately managed neonatal jaundice associated with G6PD deficiency can produce permanent neurological damage.

Acute hemolytic anemia (AHA)

G6PD-deficient subjects are at risk of developing AHA in response to three types of triggers: (i) fava beans, (ii) infections, and (iii) drugs. Typically, a hemolytic attack starts with malaise, weakness, and abdominal or lumbar pain. After an interval of several hours to 2-3 days the patient develops jaundice and dark urine, due to hemoglobinuria. The onset can be extremely abrupt, especially with favism in children. The anemia is from moderate to extremely severe, it is usually normocytic and normochromic, and it is due largely to intravascular hemolysis: hence it is associated with hemoglobinemia, hemoglobinuria and low or absent plasma haptoglobin. The blood film shows anisocytosis, polychromasia, spherocytes (Figure 3). The most typical feature is the presence of bizarre poikilocytes, with red cells that appear to have unevenly distributed hemoglobin (hemighosts), and red cells that appear to have had parts of them bitten away (bite cells or blister cells). A classical test, now rarely carried out, is supravital staining with methyl violet which, if done promptly, reveals the presence of Heinz bodies, consisting of precipitates of denatured hemoglobin, and regarded as a signature of oxidative damage to red cells (except for the rare occurrence of an unstable hemoglobin). The concentration of lactate dehydrogenase (LDH) is high as is that of unconjugated bilirubin, indicating that there is also extravascular hemolysis. The most serious threat from AHA in adults is the development of acute renal failure (this is exceedingly rare in children). Once the threat of acute anemia has passed, and in the absence of co-morbidity, full recovery form AHA associated with G6PD deficiency is the rule.

Chronic non-spherocytic hemolytic anemia (CNSHA)

A very small minority of subjects with G6PD deficiency has chronic anemia of variable severity. The patient is always a male, almost invariably develops neonatal jaundice, and in general he is investigated because of that or because of unexplained jaundice or because of gallstones later in life. Usually the spleen is moderately enlarged in small children, and subsequently it may increase in size sufficiently to cause mechanical discomfort, or hyper-

Figure 1. World distribution of polymorphic G6PD-deficient mutants. The different shadings indicate the frequency of the G6PD deficienct phenotype in the respective population. Modified from *Vulliamy T, Luzzatto L. (2003)*.

splenism, or both. The severity of anemia ranges in different patients from borderline to transfusion dependent. The anemia is usually normochromic but somewhat macrocytic, largely on account of reticulocytosis (up to 20 per cent or more). The red-cell morphology is not characteristic (hence the designation non-spherocytic). Bilirubin and LDH are increased. The bone marrow is normoblastic, unless there is superimposed folate deficiency. In CNSHA caused by G6PD deficiency, unlike in the AHA described above, hemolysis is mainly extravascular. However, the red cells of these patients are naturally also vulnerable to acute oxidative damage, and therefore the same agents that can cause acute hemolytic anemia in people with the ordinary type of G6PD deficiency will cause severe exacerbations in people with the severe form of G6PD deficiency.

Genetics

The gene encoding G6PD maps to the telomeric region of the long arm of the X-chromosome (band Xq28), physically very close to the genes for hemophilia A, dyskeratosis congenita and color blindness. The G6PD gene consists of 13 exons and spans some 18.5 kb. Structural and functional studies have revealed features of a housekeeping gene; this is in accord with the fact that G6PD is found in all cells. The X-linkage of the G6PD gene has important implications. First, as males have only one G6PD gene (i.e., they are hemizygous for this gene), they must be either normal or G6PD-deficient. By contrast, females, who have two G6PD genes, can be either normal or deficient (homozygous), or intermediate (heterozygous). Moreover, as a result of the phenomenon of X-chromosome inactivation, heterozygous females are genetic mosaics, and this in turn has clinical implications. Indeed, in most other (autosomal) enzyme deficiencies, heterozygotes are asymptomatic because cells with an enzyme level close to 50 per cent of normal are biochemically normal. However, in the case of G6PD, as a result of X-inactivation, the abnormal cells of a woman heterozygous for G6PD deficiency are just as deficient as those of a hemizygous deficient man, and therefore just as susceptible to pathology. Thus, although G6PD deficiency is still often referred to as an X-linked recessive trait, this is a mismomer because a recessive trait is, by definition, not expressed in a heterozygote: instead, G6PD deficiency is expressed both biochemically and clinically in heterozygotes. Although on average heterozygotes have less severe clinical manifestations, individual heterozygotes may develop severe AHA.

Biochemistry and pathophysiology

G6PD catalyses the conversion of glucose 6-phosphate (G6P) to 6-phosphogluconolactone, with concomitant reduction of NADP to NADPH. NADPH in turn, via glutathione reductase, produces glutathione (GSH), required for the operation of GSH peroxidase; and NADPH also stabilizes catalase: these two enzymes are able to detoxify hydrogen peroxide, which is produced from oxygen radicals (such as superoxide) whenever cells are subjected to oxidative stress. Red cells are highly exposed to such stress for two reasons. First, oxygen radicals are generated continuously from within the red cells as hemoglobin cycles from its deoxygenated to its oxygenated form. Second, red cells are often directly exposed to a variety of exogenous oxidizing agents: for instance, phagocytosing granulocytes or certain glycosides present in fava beans. The enzymatically active form of G6PD is either a dimer or a tetramer of a single protein subunit of 514 amino acids with a molecular mass of 59 096 Da. Some regions of the molecule critical for its functions have been identified because they are highly conserved in evolution. The G6P-binding site and the active center of the enzyme are located near lysine 205. Recently the three dimensional structure of G6PD has been solved (see Figure 2). In the dimer structure the two subunits are symmetrically located across a complex interface of β-sheets. The NADP binding site is near the N-terminus, and bound NADP is important for the stability of G6PD.

Since red cells have no protein synthesis, the activity of G6PD, like that of all other red cell enzymes, decreases gradually during red-cell aging. For instance, in normal blood, reticulocytes have about five times more activity than that of the oldest 10%$ of red cells. As a result, during a hemolytic attack the oldest cells, which have less residual G6PD, will be selectively destroyed. With certain G6PD variants this phenomenon can be so marked that patients tested in the post-hemolytic period may be misclassified as G6PD normal: at this time, they may prove relatively resistant to further challenge.

Although there is a decrease in G6PD activity in most tissues in G6PD-deficient individuals, this decreased activity is less marked than in red cells, and it does not seem to influence the clinical expression. Only in some cases of CNSHA is the deficiency of G6PD is so severe also in granulocytes that it becomes rate-limiting for their oxidative burst, with consequent increased susceptibility to some bacterial infections.

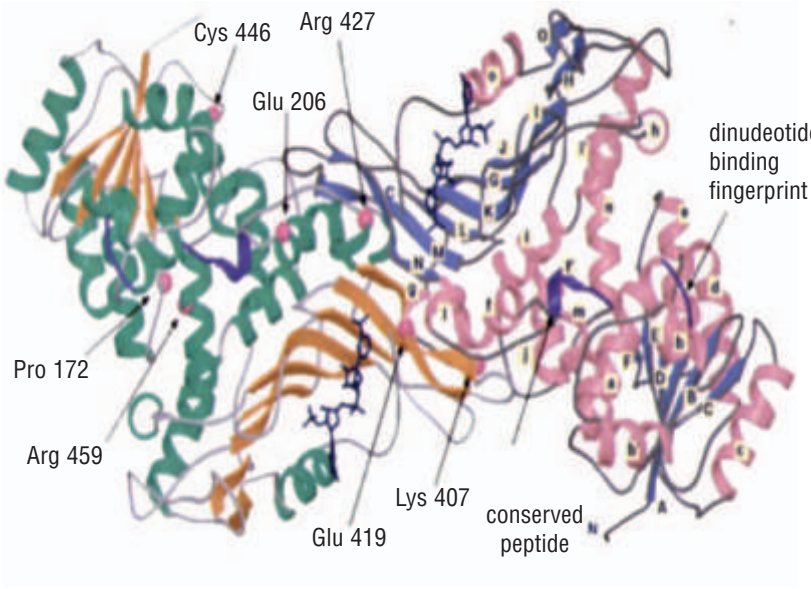

Figure 2. A model of the three-dimensional structure of the G6PD dimer. The two identical subunits are located across a dyad symmetry axis; they are not covalently linked, and their mutual interface is characterized by several β-sheet structures.

Molecular basis of G6PD deficiency

G6PD-deficient subjects have invariably been found to have mutations in the coding region of the G6PD gene (Figure 3). The current database of some 140 mutants consists, with few exceptions, of single missense point mutations, entailing single amino acid replacements in the G6PD protein. The exceptions are small deletions (of one to eight amino acids), and a few instances in which two point mutations rather than one are present (for instance, in G6PD A-β the variant most commonly encountered in Africa). In most cases these mutations cause G6PD deficiency by decreasing the in vivo stability of the protein: thus, the physiological decrease in G6PD activity that takes place with red cell aging is greatly accelerated. In some cases an amino acid replacement can also affect the catalytic function of the enzyme. The mutations underlying CNSHA form a discrete subset. This much more severe clinical phenotype can be ascribed in some cases to adverse qualitative changes (for instance, a decreased affinity for the substrate, glucose 6-phosphate); or simply to the fact that the enzyme deficit is more extreme, because of a more severe instability of the enzyme. For instance, a cluster of mutations map at or near the dimer interface, and clearly they severely compromise the formation of the dimer. In such cases the steady-state level of G6PD is so low that, even in the absence of any oxidant challenge, it becomes limiting for the survival of red cells, which may have a lifespan of between 10 and 50 days: this explains the CNSHA phenotype.

Malaria selection

G6PD is one of the best characterized enzyme protein polymorphisms in the human species. It would be quite extraordinary for a trait that causes significant pathology to spread widely and reach high frequencies in many populations without conferring some biological advantage. Indeed, clinical field studies and *in vitro* experiments strongly support the view that G6PD deficiency has been selected by *Plasmodium falciparum* malaria, by virtue of the fact that it confers a relative resistance to heterozygotes, and perhaps to hemizygotes as well, against this highly lethal infection. Different G6PD variants underlie G6PD deficiency in different parts of the world. Some of the more widespread variants are G6PD Mediterranean on the shores of

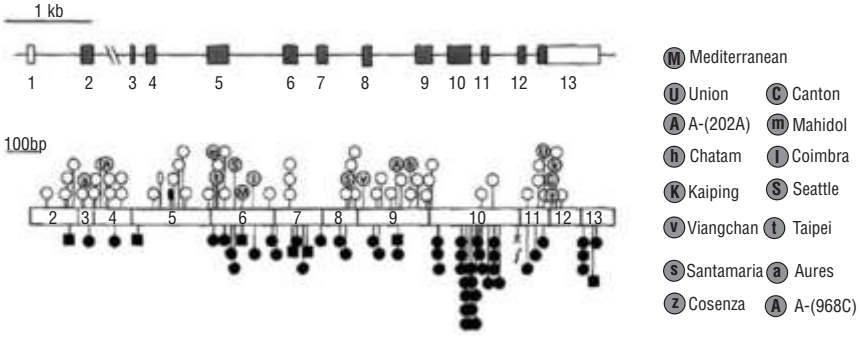

Figure 3. Genetic map of G6PD mutations. Top: diagram of the genomic structure of the human G6PD gene. Bottom: G6PD mutations are found in all of the exons. Above the line, mutations causing G6PD deficiency and therefore a risk of neonatal jaundice and AHA; mutations causing G6PD deficiency and CNSHA. Note the cluster of mutations in exon 10: these presumably affect subunit association (*see Figure 2*), and thus cause severe instability of G6PD. Only the names of some of the most widespread polymorphic mutants are shown.

this sea, in the Middle East and in India; G6PD A- in Africa and in Southern Europe; G6PD Vianchan and G6PD Mahidol in South-East Asia; G6PD Canton in China; and G6PD Union worldwide. The heterogeneity of polymorphic G6PD variants is proof of their independent origin, and it supports the notion that they have been selected by a common environmental agent, in keeping with the concept of convergent evolution (see Figure 2).

Laboratory diagnosis

When clinical and hematological findings raise the suspicion of G6PD deficiency, this must be confirmed by measuring the red cell enzyme activity. A number of screening tests (the most popular being currently the fluorescence spot test) are available for diagnostic purposes in patients who are in the steady state. However, these semi-quantitative tests are not adequate for patients in the acute hemolytic or post-hemolytic period, or for those with other complications; nor can they be expected to identify all heterozygotes. Ideally, every patient found to be G6PD-deficient by screening should then be re-tested for confirmation by a quantitative assay. In normal red cells the range of G6PD activity, measured at 30°C, is 7 to 10 IU/g Hb. In G6PD-deficient males (or homozygous females) the level of G6PD in the steady state is, by definition, less than 50 per cent of normal; but with most variants it is less than 20 per cent and with some it is practically undetectable. In heterozygous females the level is intermediate and extremely variable; in some cases the diagnosis may, therefore, be difficult without family studies or DNA analysis. However, for practical purposes it is most unlikely that a woman will have clinical manifestations if her G6PD level is more than 70 per cent of normal.

Management

Prevention

The acute hemolytic anemia of G6PD deficiency in previously screened subjects is largely preventable by avoiding exposure to triggering factors. Of course, the practicality and cost-effectiveness of screening depends on the prevalence of G6PD deficiency in each individual community. Favism is entirely preventable by not eating fava beans. Prevention of drug-induced hemolysis is possible in most cases by choosing alternative drugs.

Management of neonatal jaundice

The management of this form of jaundice does not differ from that of neonatal jaundice due to causes other than G6PD deficiency. In most cases, prompt phototherapy is highly effective and sufficient;however, when bilirubin levels are above 300 µmol/L (or even less in babies who are premature, or who have acidosis or infection), exchange blood transfusion is imperative to prevent neurological damage.

Treatment of AHA, including favism

A patient with AHA may be a diagnostic problem that, once solved, does not require any specific treatment at all; on the other hand, AHA may in some cases be a medical emergency requiring immediate action. In such cases immediate blood transfusion is imperative and may be life-saving. Hemodialysis may be necessary if there is acute renal failure.

Management of CNSHA

In general terms, CNSHA due to G6PD deficiency does not differ from that due to other causes (e.g. pyruvate kinase deficiency). If the anemia is not severe, regular folic acid supplements and regular hematological surveillance will suffice. It will be important to avoid exposure to potentially hemolytic drugs, and blood transfusion may be indicated when exacerbations occur, mostly in concomitance with intercurrent infection. In rare patients the anaemia is so severe that they must be regarded as transfusion- dependent. Since, unlike in thalassaemia, there is no ineffective erythropoiesis in the bone marrow of this type of CNSHA, a hyper-transfusion regimen aiming to suppress the bone marrow is not indicated. On the other hand, appropriate iron chelation should be instituted in patients requiring regular transfusions. Unlike in hereditary spherocytosis, there is no evidence of selective red-cell destruction in the spleen: however, in practice splenectomy has proven beneficial in severe cases. When a diagnosis of CNSHA is made, genetic counseling should be offered to the family. An important step is to establish whether the mother is a heterozygote; if she is, there is a lin 2 chance of disease in every subsequent male pregnancy. Prenatal diagnosis can be made by DNA analysis if the mutation is first identified in an affected relative.

References

1. Au SWN, Naylor CE, Gover S. Solution of the structure of tetrameric human glucose 6-phosphate dehydrogenase by molecular replacement. Acta Crystallographica 1999;D55:826-34.
2. Beutler E. Glucose 6-phosphate dehydrogenase deficiency. In Hemolytic anemia in disorders of red cell metabolism, (E Beutler, editor), p. 23. Plenum Medical; New York. 1978.
3. Beutler E. Glucose 6-phosphate dehydrogenase deficiency. N Engl J Med 1991;324:169-74.
4. Dacie JV. Hereditary enzyme deficiency haemolytic anaemias. Deficiency of glucose-6-phosphate dehydrogenase. In Haemolytic anaemias, III: The hereditary haemolytic anaemias, (editor J. Dacie), 1985; p. 364. Chirchill Livingstone, London.
5. Longo L, Vanegas OC, Patel M, Rosti V, Li H, Waka J, et al. Maternally transmitted severe glucose 6-phosphate dehydrogenase deficiency is an embryonic lethal. EMBO J 2002;21: 4229-39.
6. Vulliamy TJ, Beutler E, Luzzatto L. Variants of glucose 6-phosphate dehydrogenase are due to missense mutations spread throughout the coding region of the gene. Hum Mutat 1993; 2,159-67.
7. Vulliamy T, Luzzatto L. Glucose 6-phosphate dehydrogenase. In: Blood. Principles and practice of Hematology. Handin RJ, Lux SE.