Glucose-6-Phosphate Dehydrogenase Deficiency: Correlation between the Genotype, Biochemistry and Phenotype

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common genetic enzyme defect present in many people from African, Middle Eastern, Mediterranean and Asian countries. Individuals with the enzyme deficiency may remain asymptomatic, develop an acute haemolytic crises to infections or Fava beans, neonatal jaundice or chronic non-spherocytic haemolytic anaemia. Electrophoretic mobility may be fast, slow or normal. Over 160 mutations have been described, mostly due to single amino acid substitution. Although correlation of the genotype and biochemistry with the clinical phenotype of G6PD deficient individuals remains somewhat variable, there is better correlation among individuals presenting with chronic non-spherocytic haemolytic anaemia, which is related to the NADP structure of the enzyme.

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is the first and rate-limiting enzyme of the pentose phosphate pathway. By maintaining erythrocytic glutathione in a reduced state (GSH), the enzyme protects cells and haemoglobin against oxidative damage. Deficiency of the G6PD enzyme has been estimated to affect about 400 million people worldwide, including those living in sub-Saharan Africa, Middle-Eastern and Mediterranean countries and many parts of Asia.[1] The G6PD gene is located on the long arm of the X chromosome (Xq28), resulting in an X-linked recessive mode of inheritance.[2] While females with the disorder may be heterozygous or more rarely homozygous, males will always be hemizygous.

Classification

G6PD deficiency may be characterised in different ways, according to the clinical presentation of affected individuals, biochemical properties of the enzyme or molecular genotype at the DNA level.

The clinical manifestations of individuals with G6PD deficiency are highly variable.[3] Deficient individuals may remain asymptomatic throughout all or most of their lives, since the deficiency may have been detected during routine screening at birth. Some individuals with G6PD deficiency present with acute haemolysis induced by the ingestion of Fava beans (favism), medications, infections or other as yet unknown agents or oxidant stressors. In a few cases, the presentation is that of chronic haemolytic anaemia or neonatal jaundice. There are over 400 allelic variants of the G6PD enzyme. Variants have been classified by the World Health Organization (WHO) into 5 classes based on the residual enzyme activity (REA) and clinical manifestations.[4] Class 1 (where there is complete enzyme deficiency) is associated with chronic non-spherocytic haemolytic anaemia. Class 2 is a severe enzyme deficiency (REA <10%) that is associated with acute haemolytic anaemia, while class 3 is a moderate deficiency (REA 10% to 60%). Class 4 has very mild or no enzyme deficiency (REA >60%) and is usually asymptomatic. Class 5 has increased enzyme activity.

The biochemical properties of the G6PD enzyme include its electrophoretic mobility in pH 8.6 buffer, Michaelis constant (Km) for substrate and thermo-stability. An altered enzymatic state will have normal, fast or slow electrophoretic mobility as compared to the normal enzyme.[5]

The active G6PD enzyme is made up of 2 (dimer) or 4 (tetramer) identical sub-units. The primary structure of

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each sub-unit has a molecular weight of 59,265 daltons and consists of 514 aminoacids.[6] The protein-encoding segment of the gene consists of 13 exons spanning 18 kb long.[2] Molecular sequencing has identified over 160 mutations, most of which are the result of single base changes from missense mutations, resulting in single amino acid substitution. Very few variants are the results of in-frame deletions that consist of 1 to 8 codons.[6]

Various researchers attempted more recently to correlate the clinical phenotype and biochemical characteristics of the G6PD enzyme with molecular genotype.

Correlation

Alfinito et al[7] studied 31 G6PD deficient unrelated males in Naples, Italy, including 17 who had been referred following haemolytic crises and 14 who were asymptomatic military personnel detected through screening. The most common variant was G6PD Mediterranean (45%), followed by G6PD Seattle (26%), G6PD A^- (13%), G6PD Cassano (6%) and single cases of G6PD Maewo and G6PD Cosenza. Molecular lesions in the group with haemolytic crises showed a predominance of G6PD Mediterranean (70%). In the group of asymptomatic G6PD-deficient patients, the molecular variant most frequently found was G6PD Seattle (56%), followed by G6PD Mediterranean, Cassano, Cosenza and Neapolis.[7]

Pietrapertosa et al examined fifty-four G6PD deficient unrelated males from Apulia, Italy, for 4 mutations that were known to be more prevalent in the region.[8] The allelic frequency of G6PD Mediterranean (563CT), Seattle (844GC), A^- (202GA) and Montalbano (854GA) in their study population was 48%, 33%, 7% and 3.7% respectively. Four other subjects (7.4%) had an unknown variant. The results suggested that the Apulia population had a polymorphic G6PD molecular deficiency. Most patients (88%) with the G6PD Mediterranean variant had severe (class 2) enzymatic deficiency and normal electrophoretic mobility. Over half of those with G6PD Mediterranean also presented in haemolytic crises. The G6PD Seattle variant was equally seen in subjects with severe and moderate enzymatic deficiency. All patients with G6PD Seattle had slow electrophoretic mobility and were mostly asymptomatic. Most patients (75%) with the G6PD A^- variant had severe enzymatic deficiency together with fast electrophoretic mobility and presented in acute haemolytic crises caused by unknown agents. The G6PD Montalbano variant was associated with severe enzymatic deficiency and normal electrophoretic mobility but did not present with haemolytic crises. Pietrapertosa et al[8] concluded from their data that G6PD enzymatic activity was a poor predictive parameter of acute haemolytic crises and not correlated with clinical features.

Although enzymatic levels remained poorly correlated with acute haemolysis, there appeared to be better correlation of the genotype with phenotype in individuals who presented with CNSHA (WHO class 1). Mason and colleagues beautifully illustrated how a majority of CNSHA mutations were clustered near to the dimer interface and structural NADP molecule or deletion mutations. These mutations in the dimer surface disrupted dimer-dimer contacts between the 2 sub-units or disrupted the structure in the interface by introducing a differently charged or different sized residue.[9] Hirono et al[10] found that class 1 variants that were mutated in a small region of the molecule between residues 385Cys, 386Lys, 387Arg and 410Gly had a raised Km NADP and were extremely thermolabile in low (10 μM) NADP. The enzymes that were rendered inactive in low NADP were reactivated on exposure to high NADP, suggesting that the mutations affected NADP binding and stability.[10]

Babies with G6PD deficiency frequently develop significant hyperbilirubinemia in the first week of life, requiring phototherapy. Several authors had shown that G6PD-deficient babies developed higher serum bilirubin levels than those with normal G6PD values, even when no evidence of other factors known to cause hyperbilirubinemia was present.[11,12] Furthermore, babies with G6PD deficiency were more likely to require exchange transfusion than those without.[11]

Ainoon et al[13] studied 86 G6PD deficient Malaysian Malay male babies through DNA analysis of umbilical cord blood samples. G6PD Viangchan (871GA), Mediterranean (563CT) and Mahidol (487GA) accounted for 37%, 27% and 15% respectively of the cases studied. Of the 71 neonates who developed jaundice, 57 (80%) required phototherapy, with only 1 progressing to severe jaundice requiring an exchange transfusion. They found no significant difference in the incidence of neonatal jaundice, mean serum bilirubin level, mean age for peak serum bilirubin, percentage of babies requiring phototherapy or mean duration of phototherapy between the 3 common variants that they studied.[13]

While several studies showed variable correlation between genotype, biochemistry and clinical characteristics of G6PD deficiency, there appeared to be much better correlation between molecular structure and chronic non-spherocytic haemolytic anaemia. Residual enzyme activity was generally poorly predictive of the clinical presentation.

Reasons

Several reasons were suggested for the variable clinical manifestations of G6PD deficiency.[14] In females diagnosed with G6PD deficiency, clinical expression was related to the random inactivation of the chromatin of the G6PD-normal or G6PD-deficient chromosome, a phenomenon

first proposed by Lyon.[15] Among males with G6PD deficiency, the response of different individuals with the same mutation to a single drug dose may vary widely, depending on the acetylator status of an affected individual.[16]

The age group and dietary pattern of the G6PD deficient individual influences the clinical presentation. While a G6PD deficient adult may consume Fava beans in the diet, a newborn baby would have minimal exposure to Fava beans so that favism would not be reported in the neonatal period. Even among affected individuals with similar G6PD mutations, not all would have the same reaction towards Fava bean. It had been proposed that superimposed genetic deficiencies may account for the highly variable haemolytic response to Fava beans.[14]

Among babies with G6PD deficiency, neonatal jaundice has often been ascribed to insufficient conjugation of serum bilirubin with liver glucuronide, rarely to increased haemolysis alone. In 1996, the cause of Gilbert's syndrome was identified as a polymorphism in the promoter region of the uridine diphosphate glucuronosyl-transferase-1 (UDPGT-1) gene important for bilirubin conjugation.[17] Subsequently, it was shown that a major factor influencing bilirubin levels in babies with G6PD deficiency lay in the expression of this UDPGT-1 gene.[18, 19]

Conclusion

Correlation between the genotype, biochemical characteristics and clinical phenotype of G6PD deficient individuals presenting with acute haemolysis remains somewhat variable. Nevertheless, there is better correlation between the molecular variants and the clinical presentation of WHO class 1 individuals with chronic non-spherocytic haemolytic anaemia. This correlation has been associated with the NADP structure of the enzyme. Further study will aid in unravelling the complex interaction of molecular variants, environmental factors (such as infection and medications), oxidative stress and residual enzymatic activity on the clinical expression of G6PD deficiency in affected individuals.

REFERENCES

1. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet 2008;371:64-74.
2. Martini G, Toniolo D, Vulliamy T, Luzzatto L, Dono R, Viglietto G, et al. Structural analysis of the X-linked gene encoding human glucose-6-phosphate dehydrogenase. EMBO J 1986;5:1849-55.
3. Beutler E. Glucose-6-phosphate dehydrogenase deficiency: A historical perspective. Blood 2008 Jan 1;111:16-24.
4. Yoshida A, Beutler E, Motulsky AG. Human glucose-6-phosphate dehydrogenase variants. Bull World Health Organ 1971;45:243-53.
5. WHO Scientific Group. Standardization of procedures for the study of glucose-6-phosphate dehydrogenase. World Health Organ Tech Rep Ser. 1967;366:1-53.
6. Takizawa T, Huang IY, Ikuta T, Yoshida A. Human glucose-6-phosphate dehydrogenase: Primary structure and cDNA cloning. Proc Natl Acad Sci USA 1986;83:4157-61.
7. Alfinito F, Cimmino A, Ferraro F, Cubellis MV, Vitagliano L, Francese M, et al. Molecular characterization of G6PD deficiency in Southern Italy: heterogeneity, correlation genotype-phenotype and description of a new variant (G6PD Neapolis). Br J Hematol 1997;98:41-6.
8. Pietrapertosa A, Palma A, Campanale D, Delios G, Vitucci A, Tannoia N. Genotype and phenotype correlation in glucose-6-phosphate dehydrogenase deficiency. Hematologica 2001;86:30-5.
9. Mason PJ, Bautista JM, Gilsanz F. G6PD deficiency: the genotype-phenotype association. Blood Rev 2007;21:267-83.
10. Hirono A, Kuhl W, Gelbart T, Forman L, Fairbanks VF, Beutler E. Identification of the binding domain for NADP+ of human glucose-6-phosphate dehydrogenase by sequence analysis of mutants. Proc Natl Acad Sci U S A 1989;86:10015-7.
11. Iranpour R, Akbar MR, Haghshenas I. Glucose-6-phosphate dehydrogenase deficiency in neonates. Indian J Pediatr 2003;70:855-7.
12. Abolghasemi H, Mehrani H, Amid A. An update on the prevalence of glucose-6-phosphate dehydrogenase deficiency and neonatal jaundice in Tehran neonates. Clin Biochem 2004;37:241-4.
13. Ainoon O, Yu YH, Amir Muhriz AL, Boo NY, Cheong SK, Hamidah NH. Glucose-6-phosphate dehydrogenase (G6PD) variants in Malaysian Malays. Hum Mutat 2003;21:101.
14. Beutler E. Discrepancies between genotype and phenotype in hematology: An important frontier. Blood 2001;98:2597-602.
15. Lyon MF. Gene action in the X-chromosome of the mouse. Nature 1961;190:372-3.
16. Magon AM, Leipzig RM, Zannoni VG, Brewer GJ. Interactions of glucose-6-phosphate dehydrogenase deficiency with drug acetylation and hydroxylation reactions. J Lab Clin Med 1981;97:764-70.
17. Monaghan G, Ryan M, Seddon R, Hume R, Burchell B. Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome. Lancet 1996;347:578-81.
18. Kaplan M, Renbaum P, Levy-Lahad E, Hammerman C, Lahad A and Beutler E. Gilbert syndrome and glucose-6-phosphate dehydrogenase deficiency: A dose-dependent genetic interaction crucial to neonatal hyperbilirubinemia. Proc Natl Acad Sci 1997;94:12128-32.
19. Sampietro M, Lupica L, Perrero L, Comino A, Montemuros FM, Cappellini MD, et al. The expression of uridine diphosphate glucuronosyl-transferase gene is a major determinant of bilirubin level in heterozygous b-thalassaemia and in glucose-6-phosphate dehydrogenase deficiency. Br J Hematol 1997;99:437-9.

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