



Distribution of Mutations in Glucose-6-Phosphate Dehydrogenase Gene: A Review

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Abstract

The knowledge of possible mutations in the exons, introns and promoter regions of glucose-6-phosphate dehydrogenase (G-6-PD) gene are indispensable to improve neonatal health in Jaundice babies and prevent haemolytic episodes through appropriate drug selection in a deficient and COVID-19 infected subject. G-6-PD deficiency literature was independently searched from science direct and PubMed databases. Thirty-eight selected cross-sectional studies published between 1991 and 2024 were assessed for quality assessments with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement. The inter-rater reliability was good ($\kappa=0.76$). The study reveals that majority of the mutations were G-6-PD Africa (G202A/A376G), followed by G-6-PD Mediterranean (C563T) with occurrence among African, Asian, Europe and Middle East populations. The G-6-PD (A⁻) (A376G/G202A) variants were found among Americans and the single mutation A376G co-exists with other G-6-PD variants to produce double mutations. The review concludes that G-6-PD mutations were not found in the promoter but within the coding and non-coding regions of the gene. The observed G-6-PD (A) variant is a frequent polymorphism across the globe, and its molecular characterization could serve as a first line approach for identifying other variants.

Keywords: G-6-PD mutations, malaria control, acute haemolytic anaemia, polymorphism

Introduction

In humans, the G-6-PD gene is located on the telomeric region of the long arm of the X chromosome in position q28. This gene spans about 18 kb with 13 exons (12 bp) and 12 introns (236 bp) in size [1]. All these exons are the coding segments of DNA except the first exon which contains no coding sequence [2]. Exons are transcribed into messenger RNA (mRNA), which then serves as the template from which the amino acid sequence as well as the protein structure is determined. Introns are intervening sequences serving no apparently functional purpose [3]. The intron 2 between exons 2 and 3 is extraordinarily long, extending for 9,857 bp [2, 4].

Glucose-6-phosphate dehydrogenase gene encodes for G-6-PD enzyme that provide vital antioxidant for protection of body tissues including the red blood cells against oxidative damage via the glutathione cycle [5]. The molecular characterization of G-6-PD variants is patchy globally with varying frequency across different populations and the severity of this disease is associated with the type of molecular variants. Currently, 217 mutations of have been described to have 182 (83.9%) are single nucleotide substitutions (missense variants), 19 (8.7%) are multiple mutations (two or more substitutions), 11 (5.1%) are deletions, and five (2.3%) mutations affect the introns [6]. In West Africa, the A⁻ allele, a mild form (class III) of G-6-PD deficiency occurs in polymorphic frequencies [7, 8]. The African G-6-PD-A⁻ variant is been shown to be associated with two nucleotide substitutions occurring at nt 202 (G/A); exons 4 and nt 376 (AAT/GAT); exons 5 of G-6-PD gene [9]. Recent studies demonstrated that the enzyme activity is not affected unless mutation occurs at nucleotide position 376 mutation is present [10]. The presence of a mutation at position 202 is considered as diagnostic of G6PD A⁻ because it has never been found in isolation. The review seeks to update or make a compendium of the molecular variants described in Nigeria, mainly, in order to provide

relevant information to the health authorities who are responsible for decision-making in health matters especially in to digest 8-aminoquinolines (e.g., primaquine (PQ) and its derivatives) and some exogenous agent (e.g., Fava bean) by travelers in an effort to reduce the risk of drug-induced G-6-PD deficiency-related haemolysis and oxidative stress. Knowledge on individual G-6-PD status is of benefit especially when planning national strategies for the use of licensed drugs that may be associated to haemolysis among G-6-PD individuals, and in the management of chronic and infectious diseases including COVID-19, associated with induction of oxidative stress.

Methods

1. Literature Search Strategy

For retrieval of the observational studies, that report primary data on the G-6-PD deficiency and its molecular nature in the world and Nigeria, we searched Science Direct and PubMed between 1991 and 2024. The search terms used were "G-6-PD deficiency, Nigeria, Africa and other continent of the world, molecular nature of G-6-PD deficiency" The search was conducted by the authors.

2. Inclusion Criteria

Studies were included if they reported on the G-6-PD mutation in Nigeria and continents of the world, molecular analysis in English language, and full text was available.

3. Exclusion Criteria

Studies in duplicates and quantitative methods of G-6-PD testing were excluded from the study.

4. Outcome

The main outcome of this review was to identify molecular variance of G-6-PD, Molecular studies for G-6-PD analysis for region-specific and country-specific prevalence estimates, strategic methods of assessment and diagnostic procedures.

5. Study Selection, Quality Assessment, and Data Extraction

In this systematic review, selected studies included cross-sectional studies that met the inclusion criteria. We searched the reference lists of identified articles for studies that may not have been captured by the database searches. The search detail was given according to PRISMA guideline. The retrieved studies were screened by 2 reviewers independently. A quality-assessment of included studies was done on the basis of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. Data were extracted independently. The main search codes for quality assessment of the articles were study design, sampling, location and molecular characteristics of G-6-PD deficiency.

6. Data analysis

The descriptive data were subjected to descriptive statistics for frequency counts of G-6-PD variants and Cohen's kappa statistic for the inter-rater agreement respectively using Statistical Products for Service Solution (SPSS) Version 26.

Results

The calculation of percentage agreement across two raters using the Cohen's Kappa Statistics was done using SPSS software. The inter-rater reliability was good ($\kappa=0.76$). Initially, 112 articles were obtained. After exclusion of case control studies, duplicate studies, studies conducted on hypertensive and neonates, and studies that adopted non-molecular techniques, there were thirty-eight eligible reviews across the globe. Studies under review have indicated the use of several diagnostic approaches for G-6-PD deficiency and its allelic variants analysis as detailed in Table 1. Several biochemical methods commonly performed to exclude non-deficient subjects before molecular testing were Enzyme Analysis Assay, Fluorescence Spot-Test, Cellulose Acetate Electrophoresis, methaemoglobin reduction method, and Biochemical Characterization. The Enzyme Analysis Assay and Fluorescence Spot-Test were more frequently used for excluding presumably non-deficient individuals. For molecular studies, both single and two or more- strategic stages have been used. Specific point mutations were detected by a one-step multi-allelic specific PCR with DiaPlexC G-6-PD genotyping kit. A two-step approach after PCR technique has been performed through RFLP and DNA Sequencing; details are expanded in Table 1. The frequency of G-6-PD variants included in the current review was found to be more in Asia, followed by Africa then Middle East and America respectively (Table 2). Most of these allelic variants are missense mutations existing as single or multiple (double or more) with the rest being silent, frame shift or nonsense mutations, details are expanded in Table 2.

Discussion

The present study has indicated the use of several diagnostic approaches for G-6-PD deficiency and its allelic variants analysis. This is only achieved through proper sampling and processing with appropriate test methods *viz-a-viz* the objective of the molecular study. Molecular characterization of G-6-PD was previously done by the Polymerase Chain Reaction (PCR) restriction enzyme (RE) method. This approach allows very rapid, simple and inexpensive SNP detection within the sequences of PCR products. The PCR-

Restriction Fragment Length Polymorphism (RFLP) method is more time-consuming, especially for post-amplification steps, such as verifying the presence of PCR products by gel electrophoresis, restriction endonuclease digestion and restriction pattern visualization by gel electrophoresis. However, significant false positive results were recorded due to incomplete restriction enzyme digestion [11]. This indicates the challenge of narrower mutational detection on the gene due to its heterogeneous pattern of zygosity. There has diagnostic improvement to provide a simple and rapid alternative to PCR-RFLP which detects six different G-6-PD point mutations by a one-step multi-allelic specific PCR with DiaPlexC G-6-PD genotyping kit as adopted [12]. However, this technique is still limited to the detection of many G-6-PD mutations since its deficiency is heterogeneous.

A two-step approach after PCR technique has been performed [13, 14] through RFLP and DNA Sequencing. DNA-based techniques allow the determination of all G-6-PD genotypes independent of the enzyme activity or the ratio of deficient and normal erythrocytes in heterozygotes. Therefore, G-6-PD genotyping may be regarded as a safe technique in defining heterozygosity.

Most of these allelic variants are missense mutations existing as single or multiple (double or more) with the rest being silent, frame shift or nonsense mutations. In a state of double heterozygosity and one was homozygous for G-6-PD A⁻, the missense mutation (202A/376G/536T) has co-inherited in two females [15]. In addition, similar co-inheritance of G-6-PD missense variants was reported only once, in a mixed Indian-African origin of Oman population [16]. In Burkino Faso, double pattern of G-6-PD missense mutants' inheritance was reported by Ouattara *et al.* [17] in a female subject bearing the G-6-PDA⁻ (202A/376G) on one of the X chromosomes and G-6-PDA⁻ (376G/542T) on the other X chromosomes. Studies have shown that the African variants of G-6-PD are seen in Indian among Indo-Mauritian (of Indian origin), and Creoles (of African ancestry) [18], Italians [19] and Venezuelan population from America [20], suggesting a significant flow of G-6-PD mutated genes from Africa to India, Italy and America, details are expanded in Table 2.

Silent mutations have been shown to occur within the exonic and intronic region of the G-6-PD gene and an exceptional case that occur along the exon-intronic region. This exception is for a combination of c.1311C4T in exon 11 with IVS11 T93C (designated here as G-6-PD 1311T/93C). This combination is a special G-6-PD variant where the carrier is deficient without any changes to the protein sequence of the G-6-PD enzyme. Interestingly, some individuals with genotype 1311T/93C show significant reduction in G-6-PD enzyme level, while some individuals with this genotype present normal G-6-PD activity [21]. Studies have found silent mutations at nt 175 C>T on intron 7, nt 163 C>T on introns 8 [22], however, found silent mutation at nt 93 T>C on introns 11 [23].

A good example of nonsense mutation is the G-6-PD Georgia which was reported among the Filipino ethnic origin, resulting from changes in the nucleotide at position 1284 C/A that codes for amino acid tyrosine at position 428 Try. Sporadic variants causing chronic non-spherocytic haemolytic anaemia (CNSHA) (also known as WHO class I) occurs at a very low frequency in any part of the world [4].

This shows that such mutation was only detected in a heterozygous individual implies its lethality in the hemizygous state and the essential nature of G-6-PD for survival [24]. The large deletions and frame-shift mutations leading to the complete lacking of the G-6-PD functionality have not been observed yet [25]. Although, Small in frame deletion of several base pair (bp) with at-least 3 bp and not more than 49 bp deletions in exons and introns were reported [26].

1. Strengths and Limitations

This is the first study to update or make a compendium of the molecular variants of G-6-PD described in Nigeria. We did not consider sample size which may limit the

generalization of the results to the current review. The results reflect a gap in knowledge in the molecular studies in G-6-PD deficiency for Nigerian populations.

Conclusion

Single Nucleotide Polymorphism genotyping by PCR-Sequencing approach seems to be a must do approach due to the heterogeneity nature of G-6-PD mutation and its ability to exclude false positive results emanating from screening methods. The observed African variant (G202A/ A376G) is the commonest and widely spread polymorphism in subjects with G-6-PD deficiency across the globe and its molecular detections could serve as first line of approach for other variants.

Table 1: Studies included in the G-6-PD mutation review

| Region | Country | Reference | Strategic steps of G-6-PD assessment | SNPs mutation |
|---------|-------------------|-----------|---|--|
| Africa | Burkina Faso | [17] | PCR-GE, real time PCR | G202A/A376G; G202A/T968C; G202A/A376G/A542T |
| | Equatorial Guinea | [9] | FST, HRM, PCR-DNA sequencing and RDBH | G202A /A376G; T968C/A376G |
| | Ethiopia | [27] | CareStart G-6-PD Biosensor, PCR, DNA sequencing | G202A/A376G; G267+119C/T; G1116A; C536T |
| | Gambia | [8] | EAA, PEP-Sequenom MASS array iPLEX platform | G202A/A376G; A542T, G680T; T968C |
| | Ghana | [28] | PCR- SSOP | G202A/A376G (A) |
| | Kenya | [29] | EAA, Sequenom Mass ARRAY iPLEX platform | C202T |
| | Mauritania | [12] | CareStart G6PD deficiency rapid diagnostic test | C563T; A376G; G202A/A376G; T968C/A376G; A376G/A542T |
| | Nigeria | [30] | PCR-Sanger Sequencing | G202A/A376G |
| | Tunisia | [31] | PCR-RFLP and DNA sequencing | G202A/A376G; C563T; G1388T; G1003A; C680A; T143C; T968C/A376G; A542T; A920C; T968C; C1311T /IVS11 T93C; G1376T; A1220C;376G/C1311T/IVS11T93C |
| America | Venezuela | [20] | EAA, PCR-RFLP and direct DNA sequencing | G202A /A376G |
| | Brazil | [32] | MRM, EAA, PCR-RFLP | 202 G/A |
| | San Diego | [33] | BC, DNA Sequence Analysis | G637T; G1178A; C1089A |
| | Mexico | [10] | PCR-GE | A376G/T968C |
| | Bangladesh | [34] | EAA, FST, PCR and Sanger sequencing | C131G; G487A; G949A |
| Asia | China | [14] | PCR-Sequencing | G1388T; G1399A |
| | | [25] | EAA, PCR-GE, sequencing | C1311T |
| | | [35] | EAA, Microarray assay, sequencing | A95G; A371G; G392T; C519G; C1311T; G1376T; G1388A; G 487A; A493G; C592T; C1024T; C1360T |
| | | [21] | NTPS, ARMS, PCR-RFLP/SSCP, dHPLC, sequencing | C274T |
| | | [36] | EAA, FST, qPCR-Sequencing | G1376T; G1388A; A95G; C1024T; G871A |
| | India | [18] | FST, CAE, PCR-SSCP/RFLP, sequencing | C131G; C563T; G949A; G487A; G844C; C1360T; T323A |
| | Indonesia | [37] | MRM, PCR-Sequencing | T383C |
| | | [38] | EAA, PCR-Sequencing | T383C; C592T; G871A; G1003A; G1388A |
| | Malaysia | [39] | EAA, dHPLC, sequencing | C1311T/T93C |
| | | [40] | EAA, PCR-RFLP | G487A; G871A |
| | Pakistan | [41] | PCR-RFLP, sequencing | C563T; C1311T |
| | Singapore | [26] | EAA, MPTP, sequencing | G1376T; G871A; G1388A; T383C; C563T; A95G; G487A; C131G; C592T; G1003A; C25T; A209G; G392T; T517C; C1024T |
| | Taiwan | [42] | EAA, PCR-Sequenom® iPLEX platform | G1376T; C1360 T; G1388A; G871A; C1024T; G392T |
| | Thailand | [43] | WST-8/1-methoxy PMS method, PCR-RFLP/sequencing | G487A; G871A; 1311C>T and IVS11 93T>C; A95G; G1388A; G949A |
| | | [44] | MRM, PCR-SSCP/GE, sequencing | G7A; A95G; T10148G; C11763T; C13184T; G13031A C10170T; C13714T |
| | | [45] | FST, EAA, PCR-SSCP, sequencing | G487A; G392T; G871A; A595G; G844C; C563T |
| Europe | Italy | [19] | BC, PCR- SSCP/RFLP | C563T; G202A/ A376G; C592T; G844C; G854A; G1347C G1376C; A634G; C1360T |
| | | [6] | EAA, CAE, PCR-RFLP/SSCP, sequencing | C563T; A1342G; G1347C; C1360T; G1376C; A634G; G844C; G854A; G1246A G202A/A376G |
| Middle | Egypt | [46] | MRM, PCR-RFLP | C563T; C1311T |

| | | | | |
|------|---------------------|------|---------------------------------------|---|
| East | | | | |
| | Iran | [13] | PCR-RFLP | C563T; G1003A; G1376C |
| | Pakistan | [47] | EAA, PCR-RFLP, sequencing | G973A; C563T; A1003G; G1376C; C131G |
| | Saudi Arabia | [48] | EAA, PCR-Sequencing | T143C; C563T; A634G; G871A; G1478A G202A/A376G |
| | | [49] | EAA, PCR-RFLP, sequencing | C563T; G1003A; T143C; C1311T/T93C |
| | United Arab Emirate | [50] | FST, EAA, PCR-RFLP, dHPLC, sequencing | C563T; A95G; G392T; G487A; A 493G; C592T; G871A; C1024T; C1360T; G1376T; G1388A; C1311T |

EAA=Enzyme Analysis Assay, PCR=Polymerase Chain Reaction, SSOP= Sequence- Specific Oligonucleotide Probe, FST=Fluorescence Spot-Test, CAE=Cellulose Acetate Electrophoresis, RFLP= Restriction Fragment Length Polymorphysm, MRM= methaemoglobin reduction method, BC=Biochemical Characterization, MMCA = multicolor melting curve analysis, IVS (intervening sequence), dHPLC=Denaturing HighPerformance Liquid Chromatography. HRM=high-resolution melting assay, RDBH=reverse dot blot hybridization. NTPS=Nitriblue tetrazolium paper strip, ARMS=amplified of refractory mutation system. CT= Cytochemical test. GE=Gel electrophoresis. MPTP=

Table 2: Overview of G-6-PD variants in the studies included.

| Continents | G-6-PD mutation variants | | | | | | | | | |
|-------------|--------------------------|-------|--------|-------|--------|---------|---------|---------|---------|---------|
| Africa (9) | C202T | A376G | T542A | A542T | C680A | G680T | T968C | A968G | G1116A | |
| America (5) | G202A | G637T | C1089A | G117A | | | | | | |
| Asia (16) | G7A | C25T | A209G | C274T | T323A | A371G | T383C | C406T | T517C | C519G |
| | A595G | G134T | G1376T | G138T | G1399A | T10148G | C10170T | C11763T | G13031A | C13184T |
| | C13714T | | | | | | | | | |
| Europe (2) | G854A | G105T | G1246A | A134G | G1347C | G1360A | | | | |
| ME (6) | T143C | G973A | A1003G | G147A | | | | | | |

Numbers in parentheses indicate the frequency of studies reviewed. G-6-PD= glucose-6-phosphate dehydrogenase. A=adenine. G=guanine. C=cytosine. T=thymine. ME=Middle East

Table 3: Single Nucleotide Polymorphism of G-6-PD deficiency in the studies included separated by continents.

| Nucleotide changes across the G-6-PD gene | | | | | | |
|---|--------------------------------|-------------------------------|----------------------------|---------------------------|--------------------------|--|
| Single mutant | A95G ^{ce} | C131G ^{bee} | G392T ^{ce} | G487A ^{bee} | A493G ^{ce} | C536T ^{abce} C592T ^{cde} |
| | A634G ^{de} | G871A ^{ce} | G844C ^{cd} | G949A ^{bc} | G1003A ^{ce} | C1024T ^{ce} C1311T ^{cde} |
| Double mutant | C1311T/T93C ^{ace} | G202A/A376G ^{abcde} | G202A/T968C ^{abc} | G267+119C/T ^{ab} | T968C/A376G ^a | |
| Multiple mutant | G202A/A376G/A542T ^a | 376G/C1311T/T93C ^a | | | | |

G-6-PD= glucose-6-phosphate dehydrogenase. A=adenine. G=guanine. C=cytosine. T=thymine. G-6-PD mutations with superscript (^{a b c d e}) in rows designates continents and the superscripts denotes, a=Africa. b=America. c=Asia. d=Europe. e=Middle East.

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