Brief Report

Glucose-6-Phosphate Dehydrogenase Deficiency in Female Octogenarians, Nanogenarians, and Centenarians

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Background. Age-related skewing of X-chromosome inactivation leading to glucose-6-phosphate dehydrogenase (G6PD) deficiency in elderly women in a population with prevalent G6PD gene mutations was investigated.

Methods. G6PD activity was measured biochemically. G6PD mutations were detected by polymerase chain reaction (PCR) and allele-specific extension, and analyzed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry and Sequenom MassARRAY. X-chromosome inactivation was quantified by semiquantitative PCR for the HUMARA gene, before and after HpaII digestion.

Results. In 173 women (median age: 90 years; range, 80–107 years), 18 heterozygotes for G6PD mutations were identified. Three heterozygotes were G6PD deficient, owing to skewed X-chromosome inactivation affecting the wild-type allele. Fifteen heterozygotes, with skewing apparently affecting the mutant alleles, had normal but significantly lower G6PD levels. At 1.73%, G6PD deficiency was significantly more frequent than expected from population screening at birth.

Conclusion. Due to skewed X-chromosome inactivation, elderly women in populations with prevalent G6PD mutations are at risk of G6PD deficiency.

GLUCOSE-6-phosphate dehydrogenase (G6PD) deficiency is the most common inherited red cell enzymopathy, affecting an estimated 400 million people (1,2). It is an X-linked recessive disorder, so that the majority of G6PD-deficient patients are male, with females affected only if they are homozygous or double heterozygous for G6PD mutants. G6PD deficiency results in impaired conversion of G6P to 6-phosphogluconate, a step critical in the generation of (NADPH) required to maintain a pool of reduced glutathione. Therefore, red blood cells deficient in G6PD have a reduced capacity of handling oxidizing stress by reduced glutathione. Typically, in G6PD-deficient patients have a reduced capacity of handling oxidizing stress by reduced glutathione. Typically, in G6PD-deficient patients exposed to oxidizing drugs, including most of the antimalarial agents, some sulfur drugs, and urinary antibiotics (nalidixic acid and nitrofurantoin), a severe intravascular hemolysis may ensue. G6PD deficiency is prevalent in American and African blacks, and people from the Mediterranean regions. In Southern China, 4.47% of men are affected (3). The predominant G6PD gene mutations in this population are World Health Organization type II mutations (4). Deficient males are therefore largely asymptomatic. Hemolysis does not occur spontaneously, but can be provoked by oxidative stress and infection.

All newborns in Hong Kong are screened for G6PD deficiency. The incidence of G6PD deficiency in female newborns was 0.27% (5). As G6PD deficiency is an X-linked recessive disorder, female heterozygotes for G6PD mutations are expected to have normal G6PD levels. Deficient females are therefore either double heterozygotes or homozygotes for G6PD mutations. Unexpectedly, however, severe hemolysis related to G6PD deficiency had been observed in adult heterozygous females who were not biochemically deficient at birth (6). This phenomenon might be explained by preferential inactivation (lyonization) of the X chromosome. X-chromosome inactivation may occur in a stochastic fashion. However, recent experimental evidence suggests that, in some situations, there may be genetic determinants controlling the pattern of X-chromosome inactivation (7,8). The consequence of skewed inactivation of the X-chromosome bearing the wild-type allele is expression of the mutant allele. Because the skewing of X-chromosome inactivation has been reported to increase with age (9,10), elderly women heterozygous for G6PD mutations have a theoretical chance of presenting with biochemical G6PD deficiency.

To address whether elderly women in a population with a high prevalence of G6PD mutations are at risk of G6PD deficiency, we studied a cohort of Chinese women older than 80 years, to define the effect of age on G6PD activity.

MATERIALS AND METHODS

Blood Samples

Consecutive Chinese women older than 80 years with no hematological problems or blood transfusion for at least 3 months were studied. None of the studied participants had...
Efficient of NADPH, the hemoglobin concentration, and the rate of increase in absorbance, the millimolar extinction coefficient at 340 nm. The G6PD enzyme activity was calculated from the G6P at 540 nm absorbance. After incubation with NADP and bin concentration was estimated with Drabkin’s solution.

Briefly, red blood cells were hemolyzed, and the hemoglobin concentration was estimated with Drabkin’s solution. The G6PD enzyme activity was assayed by standard methods (11). Glucose-6-phosphate dehydrogenase (G6PD) activity and genotype. Median G6PD activity was assayed by standard methods (11).

Both alleles H1 and H2 of G6PD were reduced to the same extent after HpaII digestion, showing absence of skewing of X-chromosome inactivation. Arrow shows near complete digestion of the XE169 locus by HpaII prior to polymerase chain reaction (PCR). Skewed: Skewing of X-chromosome inactivation in an elderly woman. The H1 allele showed a slightly better amplification before HpaII digestion. A factor of 1.4 had to be applied to the H2 allele as a correction for differential amplification efficiency. After HpaII digestion, the H2 allele was much reduced as compared with the H1 allele. The skewing of inactivation was toward the H1 allele, with methylation resulting in resistance to HpaII digestion. The H2 allele on the active X chromosome was unmethylated and susceptible to HpaII digestion, which prevented PCR amplification. The extent of skewing could be calculated from the respective areas of the PCR products of the H1 and H2 alleles, taking into account the differential amplification efficiency. Median G6PD activity in patients heterozygous for G6PD mutants was significantly lower than that in patients with wild-type alleles only.

a personal or family history of hemolysis or adverse reactions to oxidizing drugs. The protocol was approved by our institutional review board.

G6PD Phenotype and Genotype

G6PD activity was assayed by standard methods (11). Briefly, red blood cells were hemolyzed, and the hemoglobin concentration was estimated with Drabkin’s solution at 540 nm absorbance. After incubation with NADP and G6P at 37°C, the change of absorbance was determined at 340 nm. The G6PD enzyme activity was calculated from the rate of increase in absorbance, the millimolar extinction coefficient of NADPH, the hemoglobin concentration, and the dilution factor, and was standardized with in-house normal and abnormal controls. Samples were screened for the seven mutations accounting for the majority of G6PD mutations in Chinese people (1376G–T, Canton; 1388G–A, Kaiping; 95A–G, Gaozhou; 871G–A, Viangchan; 1024C→T, Chinese 5; 1360C→T, Union; 392 G→T, Chinese 4). Primers for polymerase chain reaction (PCR) and allele-specific extension were designed by MassARRAY Assay-Design software (Sequenom, San Diego, CA). Sequences of the primers are available on request. DNA was amplified by primers flanking the targeted sequence, followed by dephosphorylation and allele-specific primer extension. The extension products were purified, loaded into a 384-format SpectroChip (Sequenom), and subjected to matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry. The resultant data were analyzed by the Sequenom MassARRAY system.

X-Chromosome Inactivation

X-chromosome inactivation was studied by semiquantitative PCR for a polymorphic site on the human androgen receptor (HUMARA) gene (6,12). The HUMARA gene on the X chromosome contains a highly polymorphic region of CAG repeats (n = 13–31), which enables the paternal and maternal alleles to be discriminated. In the inactivated X chromosome, an intragenic HpaII site is methylated, leading to abrogation of the restrictive site. However, in the active X chromosome, the HpaII site is intact. Therefore, prior digestion with HpaII will cleave the HUMARA gene in the active X chromosome, preventing amplification of the gene by PCR. The XE169 gene, located also on the X chromosome, contains an unmethylated HpaII site whether or not the X chromosome is inactive. It is amplified also to control for the completeness of HpaII digestion. DNA was treated overnight with and without HpaII. The HUMARA locus was then amplified by PCR with FAM-labeled primers. PCR products were analyzed on an automated DNA sequence analyzer (PE Biosystems, Foster City, CA). The percentage of X-chromosome inactivation was estimated from the change in PCR products from the two alleles (I and II) when the starting DNA was HpaII-digested (Figure 1A). The degree of skewing was obtained by 1 – (area II × E / area I + area II × E), where areas I and II referred to the total areas bound by the amplification tracings of alleles I and II in the electropherographs, and E is the ratio of amplification of allele I to allele II without HpaII digestion, which corrected for a possible differential PCR amplification (6).

RESULTS

G6PD Phenotype and Genotype

One hundred seventy-three individuals were studied, at a median age of 90 years (range: 80–107 years). G6PD deficiency was found in 1.73% of cases (three patients). This frequency of G6PD deficiency was significantly higher than that of 0.27% in female newborns (p = .013, Fischer’s exact test; relative risk: 6.3, 90% confidence interval: 2.02–19.9). Mutation analysis identified 18 persons (10.4% of cases) with heterozygous G6PD mutants (Table 1). The mutants included Canton (n = 8), Kaiping (n = 5), Gaozhou (n = 3), and abnormal controls. Samples were screened for the seven mutations accounting for the majority of G6PD mutations in Chinese people (1376G→T, Canton; 1388G→A, Kaiping; 95A→G, Gaozhou; 871G→A, Viangchan; 1024C→T, Chinese 5; 1360C→T, Union; 392 G→T, Chinese 4). Primers for polymerase chain reaction (PCR) and allele-specific extension were designed by MassARRAY Assay-Design software (Sequenom, San Diego, CA). Sequences of the primers are available on request. DNA was amplified by primers flanking the targeted sequence, followed by dephosphorylation and allele-specific primer extension. The extension products were purified, loaded into a 384-format SpectroChip (Sequenom), and subjected to matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry. The resultant data were analyzed by the Sequenom MassARRAY system.
and Viangchan (n = 2). The overall frequency of mutant alleles of this population was 5.2%, which was comparable with that of 4.47% in male newborns in our population (p = .44, \( \chi^2 \) test). The median age of the heterozygotes was comparable to that of individuals with only wild-type alleles (90 vs 89 years, p = .51; Wilcoxon Signed-Rank test).

**Relationship Between G6PD Genotypes and Phenotypes**

All three biochemically G6PD-deficient participants were heterozygotes. Although the other 15 heterozygotes had normal G6PD activities, their median G6PD level (8.3 IU/g hemoglobin, range 6.7–19.1) was still significantly lower than that of participants with wild-type alleles (median: 11.8 IU/g hemoglobin, range: 6.6–23.9) (p = .022, Wilcoxon test) (Figure 1B).

**X-Chromosome Inactivation**

There were varying degrees of skewing of X-chromosome inactivation for the whole cohort, ranging from 0% to 73% (median: 44%). The skewing was used to deduce a calculated G6PD level for heterozygotes based on the formula: estimated G6PD activity (IU/g hemoglobin) = % skewing \( \times \) 0.38 (mean G6PD activity of deficient men in our population) + (1 – % skewing) \( \times \) 11.7 (mean G6PD activity of 155 individuals with only wild-type alleles in this study). Details of the derivation of the formula have been previously published (5). Two calculated values could be obtained, depending on whether the skewing was presumed to be towards the wild type or the mutant allele (Table 1).

Table 1. Genotypic and Phenotypic Features of 18 Elderly Chinese Women Heterozygous for a Glucose-6-Phosphate Dehydrogenase (G6PD) Gene Mutant

<table>
<thead>
<tr>
<th>Age</th>
<th>G6PD Mutant</th>
<th>% Skewing</th>
<th>Wild-Type</th>
<th>G6PD Level Deduced From Skewing of Inactivation</th>
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<tbody>
<tr>
<td>1</td>
<td>87</td>
<td>1376</td>
<td>1.3</td>
<td>79.3</td>
</tr>
<tr>
<td>2</td>
<td>99</td>
<td>1376</td>
<td>3.6</td>
<td>70.8</td>
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<td>3</td>
<td>89</td>
<td>95</td>
<td>4.7</td>
<td>73.7</td>
</tr>
<tr>
<td>4</td>
<td>91</td>
<td>871</td>
<td>6.7</td>
<td>83.3</td>
</tr>
<tr>
<td>5</td>
<td>92</td>
<td>1376</td>
<td>7.1</td>
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<td>6</td>
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<td>83</td>
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<tr>
<td>8</td>
<td>80</td>
<td>1388</td>
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<td>9</td>
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<td>18</td>
<td>97</td>
<td>1388</td>
<td>19.1</td>
<td>69.0</td>
</tr>
</tbody>
</table>

Notes: *G6PD level (IU/g hemoglobin) was calculated according to the formula: % skewing \( \times \) 0.38 (mean activity of deficient men in our population) + (1 – % skewing) \( \times \) 11.7 (mean activity of 155 individuals with wild-type alleles only in this study). Details of the derivation of the formula can be found in (5).

- 871: ViangChan 871G
- 95: Gaozhou 95A
- 1376G→A
- 1376G→T
- 1388: Canton 1388G
- 871: GuangDong 871G
- 871: ViangChan 871G
- 95: Gaozhou 95A

Discussion

In populations with prevalent G6PD mutations, the prescription of drugs with oxidizing properties to male patients will often be preceded by a biochemical assay for G6PD. In contrast, such a precaution is rarely taken when the patients are women, as the double heterozygosity and homozygosity frequencies for G6PD mutations are presumed to be very low (13). Although this presumption may work in young women, the same may not be true in elderly women, where skewing of X-chromosome inactivation may occur, leading potentially to clinical manifestations of X-linked recessive genetic diseases (14). In fact, in this study we confirmed previous observations that skewing of X-chromosome inactivation occurred frequently in elderly women (10).

Another intriguing observation is the apparent selective inactivation of the X-chromosome with the mutant allele, thus favoring the expression of the wild-type allele, in the majority of heterozygotes. If X-chromosome inactivation is an entirely
random event, our observations imply that hematopoietic progenitors expressing preferentially the wild-type allele might have a survival advantage. This proposition is supported by previous experimental observations in heterozygotes with the more severe type 1 G6PD deficiency (18). In fractionated blood cell types of erythroid, myeloid, and lymphoid lineages of these individuals, there was a significant excess of G6PD-normal cells, suggesting preferential selection in vivo (16). Furthermore, cells deficient in G6PD showed increased apoptosis in vitro (19,20). There was also evidence that G6PD-normal erythrocytes might be evolutionally selected for (21). Therein, there was a significant excess of G6PD-normal red cells ameliorates to some extent the problem as predicted by the prevalence of G6PD mutations. However, the influence on health care is not diminished, as the frequency of G6PD deficiency in elderly women, at 1.73% in the current study, is still highly significant clinically.

The increase in G6PD deficiency with age will have to be shown in larger numbers of persons in other populations, given the relatively small number of affected persons observed in this study. Furthermore, we have studied persons older than 80 years, because the frequency of skewing of the X chromosome starts to increase sharply in women older than 70–80 years (22), and our previous study showed that an increase of G6PD deficiency due to skewing occurred mostly in women older than 70 years (6). A study of women aged 60 years [when skewing starts to rise (22)] to 80 years will be needed to define the risk in this age group. To validate the clinical significance of our observations, a systemic study investigating the response to oxidizing drugs in elderly women deficient in G6PD due to X-chromosome skewing will be needed. Alternatively, elderly women with adverse reactions to oxidizing drugs can be studied to determine whether they have significantly increased frequencies of G6PD deficiency. Although both topics are outside the scope of this report, they will be of interest to future studies. Finally, with the high frequency of G6PD mutations in many Asian, Mediterranean, and African populations (1,2) and increasing longevity, potential heterozygous elderly women at risk of age-related G6PD deficiency are numerous, constituting a worldwide health problem. Physicians should be aware of this risk, and it will be prudent to check the G6PD status of elderly women of these populations before prescribing drugs with oxidizing properties.

ACKNOWLEDGMENTS

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This article is dedicated to the memory of Veronica Lam, PhD, who died tragically in the South Asian tsunami disaster of December 26, 2004. We thank Professors Kathryn Cheah and Paul Tam for their support and encouragement.

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REFERENCES


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