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The First Case of a Class I Glucose-6-phosphate Dehydrogenase Deficiency, G6PD Santiago de Cuba (1339 GA), in a Chinese Population as Found in a Survey for G6PD Deficiency in Northeastern and Central China

Jichun Wang*

Hiroyuki Matsuoka[†]

Makoto Hirai[‡]

Ling Mu**

Liandi Yang^{††}

Enjie Luo^{‡‡}

*Department of Pathogen Biology, China Medical University,

[†]Division of Medical Zoology, Department of Infection and Immunity, Jichi Medical University,

[‡]Division of Medical Zoology, Department of Infection and Immunity, Jichi Medical University,

**Shenyang Infectious Disease Hospital,

^{††}Hubei Center for Disease Control and Prevention,

^{‡‡}Department of Pathogen Biology, China Medical University,

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Jichun Wang, Hiroyuki Matsuoka, Makoto Hirai, Ling Mu, Liandi Yang, and
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Abstract

In Liaoning Province in northeastern China, we found a G6PD-deficient patient at the age of 3. By the classification of the World Health Organization, this patient was categorized as class I (very severe G6PD deficiency). When we investigated the G6PD gene of the patient, we found that he had a replacement of G to A at nucleotide 1339. As a result, the amino acid at position 447 should change from Gly to Arg. This replacement is known as G6PD Santiago de Cuba, because it was first discovered in a Cuban boy who showed heavy chronic anemia. Today, 28 G6PD variants have been reported in the Chinese population, and all are categorized as class II (severe deficiency) or class III (mild deficiency); in class II or III deficiency, anemia is not present in daily life, but hemolytic attack can occur when the carrier ingests certain oxidative medicines or foods. This is the first report of a G6PD-deficient Chinese patient in the category of class I. We intended to find other G6PD-deficient cases in northeastern China and tested several hundred blood samples, but no cases of G6PD deficiency were found (0/414). In central China, where falciparum malaria was endemic from the 1950s to 1970s, we found two G6PD-deficient cases (2/27) and the other members from their families whose variant type was G6PD Kaiping (1388GT), which is a common variant in the Chinese population.

KEYWORDS: hemolytic anemia, Chinese, glucose-6-phosphate dehydrogenase, G6PD Santiago de Cuba, malaria

Original Article

The First Case of a Class I Glucose-6-phosphate Dehydrogenase Deficiency, G6PD Santiago de Cuba (1339 G > A), in a Chinese Population as Found in a Survey for G6PD Deficiency in Northeastern and Central China

Jichun Wang^{a*}, Hiroyuki Matsuoka^b, Makoto Hirai^b,
Ling Mu^c, Liandi Yang^d, and Enjie Luo^a

^aDepartment of Pathogen Biology, China Medical University, Shenyang 110001, China,

^bDivision of Medical Zoology, Department of Infection and Immunity, Jichi Medical University, Shimotsuke, Tochigi 329-0498, Japan, ^cShenyang Infectious Disease Hospital, Shenyang 110006, China, and

^dHubei Center for Disease Control and Prevention, Wuhan 430079, China

In Liaoning Province in northeastern China, we found a G6PD-deficient patient at the age of 3. By the classification of the World Health Organization, this patient was categorized as class I (very severe G6PD deficiency). When we investigated the *G6PD* gene of the patient, we found that he had a replacement of G to A at nucleotide 1339. As a result, the amino acid at position 447 should change from Gly to Arg. This replacement is known as G6PD Santiago de Cuba, because it was first discovered in a Cuban boy who showed heavy chronic anemia. Today, 28 G6PD variants have been reported in the Chinese population, and all are categorized as class II (severe deficiency) or class III (mild deficiency); in class II or III deficiency, anemia is not present in daily life, but hemolytic attack can occur when the carrier ingests certain oxidative medicines or foods. This is the first report of a G6PD-deficient Chinese patient in the category of class I. We intended to find other G6PD-deficient cases in northeastern China and tested several hundred blood samples, but no cases of G6PD deficiency were found (0/414). In central China, where falciparum malaria was endemic from the 1950s to 1970s, we found two G6PD-deficient cases (2/27) and the other members from their families whose variant type was G6PD Kaiping (1388G > T), which is a common variant in the Chinese population.

Key words: hemolytic anemia, Chinese, glucose-6-phosphate dehydrogenase, G6PD Santiago de Cuba, malaria

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most frequent hereditary abnormalities. The gene *G6PD* is distributed in 13 exons on the X-chromosome, and the length of the

open reading frame is 1545 bases [1]. Almost all cases of G6PD deficiency are caused by one amino-acid change due to a point mutation of the genomic DNA, and about 140 molecular abnormalities of the G6PD genotype have been identified [2]. As an enzyme, the monomer of G6PD consists of 515 amino acids; 2 monomers form a homo-dimer attaching at the site of the dimer interface. Once the amino acid sub-

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*Corresponding author. Phone:+86-24-23256666; Fax:+86-24-23262634
E-mail:jichunwang@hotmail.com (J. Wang)

stitution occurs near the dimer interface, G6PD activity becomes very low. The dimer formation may be important for the function of the active enzyme [3]. The World Health Organization (WHO) categorizes G6PD-deficiency variants into 3 classes according to their severity. The class I variant shows very low G6PD activity (less than 1% of that in a normal individual), and carriers of these genes suffer from heavy anemia because hemolysis occurs chronically. The class II variant shows low G6PD activity (less than 10% of residual enzyme activity) and does not pose as serious a problem in daily life. Hemolytic attack occurs only when the carrier ingests certain foods or medicines. The class III variant shows 10–60% of residual enzyme activity and hemolysis occurs only in rare cases [4]. Females, but not males, can be heterozygous for the class I or class II G6PD variants, and such carriers are sometimes categorized into class III, because half of their red blood cells lack the G6PD enzyme activity, while the other half have full activity.

We have previously investigated variants of G6PD deficiency in Asian countries [5–12], where this abnormality is highly prevalent in malaria-endemic areas. We have introduced a rapid diagnosis method for malaria [13] and rapid G6PD-deficiency tests [14–16] in malaria-endemic areas. Using these methods, patients are notified of the results of blood examination within 30 min and are able to receive anti-malarial medicine including primaquine [17, 18]. Primaquine can kill gametocytes, the sexual stage of malaria parasites, which are the cause of malaria transmission to mosquitoes. However, when G6PD-deficient persons take primaquine, a hemolytic attack can occur. Without G6PD, erythrocytes cannot prepare a sufficient amount of reduced pyridine nucleotide and reduced glutathione, and cannot prevent oxidant attack by primaquine. Thus, primaquine should not be administered to malaria patients before confirming their G6PD activity. In our previous studies, we have diagnosed more than 200 G6PD-deficiency cases and found 15 variants of G6PD deficiency in Southeast Asian countries. These G6PD variants were all categorized as class II. Among them, we discovered three new G6PD variants, which we designated G6PD Surabaya [5], G6PD Bajo Maumere [10], and G6PD Bao Loc [11].

Numerous studies have investigated G6PD defi-

ciency in the Chinese population. Twenty-two molecular variants were reported from the southern part of China, which includes many malaria-endemic areas [19–22]. Some other variants were reported from Chinese populations in Taiwan [23–26], Singapore [27], Malaysia [12, 28], Indonesia [5] and Hawaii [29]. These G6PD variants were all categorized as class II or class III. In this report we present the first Chinese case of G6PD deficiency categorized as class I. We also conducted a G6PD test in northeastern and central China to investigate the G6PD variants present in these areas.

Materials and Methods

This study was approved by the Ethical Committees of China Medical University, Jichi Medical University, Shenyang Infectious Disease Hospital, and Hubei Center for Disease Control and Prevention.

A case report. A 3-year-old boy who showed heavy anemia was admitted to Shengjing Hospital of China Medical University, Shenyang. His erythrocyte number was $1.25 \times 10^{12}/L$, his hemoglobin concentration was 45 g/L, and his total bilirubin level was 24.3 $\mu\text{mol}/L$. He had had infant jaundice since birth, and this condition was still present upon admission. He was first diagnosed with hemolytic anemia, and then with G6PD deficiency, because his G6PD activity was found to be less than 1% of the normal level. By the classification of the World Health Organization [4], he was categorized as class I. Artificial nutrition and recuperation led to a recovery of his scores (RBC: $2.98 \times 10^{12}/L$; Hb: 93 g/L), and he was discharged. We asked his family members for permission to analyze their G6PD activity and sequence their *G6PD* gene, and received informed consent (Fig. 1A).

Participants in the G6PD test. We asked outpatients of Shenyang Infectious Disease Hospital, Shenyang, if they would agree to be tested for G6PD activity upon providing a venous blood sample for other purposes, e.g., for an RBC count, a WBC count, or blood sugar levels. From patients who provided informed consent, we collected about 10 μl of blood onto a filter sheet, which was then dried and stored at -20°C . There were 414 males and 105 females who agreed to be tested for G6PD activity. In

addition, we visited a junior high school in Suizhou, which is in Hubei Province in central China, and received informed consent from the principle and students to test G6PD activity. Fifty students (27 males and 23 females) participated in this test. Five microliters of blood was taken from the ear lobe and put in a tube containing reagents for the G6PD test, which Hirono *et al.* described [15]. Finally, we were introduced to 2 students' families by the Suizhou Center for Disease Control and Prevention. Members of the 2 families had been diagnosed with G6PD deficiency and had previously had episodes of hemolysis (Fig. 1B and 1C).

G6PD test. Fujii's method was used at Shenyang Infectious Disease Hospital and Shengjing Hospital [14], and Hirono's method was used at the junior high school in Suizhou [15]. A 10 μ l blood sample was taken from each D6PD-deficient individual and kept on a filter sheet for analysis of the *G6PD* gene.

Analysis of the *G6PD* gene. The filter papers containing blood samples were dried, sent to Jichi Medical University, and kept at -20°C . DNA was eluted from the filter paper by heating at 80°C for 10 min in DNA extraction buffer. DNA extraction and purification were conducted using a DNA purification kit according to the manufacturer's instruction (Amersham Pharmacia Biotech, Buckinghamshire, UK). Since genomic *G6PD* consists of 13 exons, we prepared primers for these exons [11], amplified each

exon by PCR, and read the DNA sequence (ABI PRISM 310; Applied Biosystems, Foster City, CA, USA). Both strands of each exon were sequenced. To indicate the mutation point, the nucleotide number of the cDNA sequence was used.

Results

We read all the exons encoding the *G6PD* gene of the first patient and found a replacement of G to A at nucleotide 1339 in exon 11 (Fig. 2A). As a result, the amino acid was expected to change from Gly to Arg at position 447 in the molecule. The sequencing results also confirmed that his mother was a heterozygote of G6PD Santiago de Cuba (Fig. 2B) and his grandfather was a hemizygot of the same variant.

All blood samples collected from the 414 males and 105 females at the Shenyang Infectious Disease Hospital in Liaoning Province showed normal G6PD activity. The rate of G6PD deficiency was thus 0% (0/414).

In Hubei Province in Central China, we found 2 males with G6PD deficiency among 50 students (7.4%; 2 in 27 males). As described above, we also collected blood samples from 2 families with G6PD deficiency and analyzed their *G6PD* genes. After providing the informed consent, 5 individuals from the 2 families participated. We found that all of these individuals had the G6PD Kaiping (1388G > T) variant, which is one of the most frequent types of G6PD

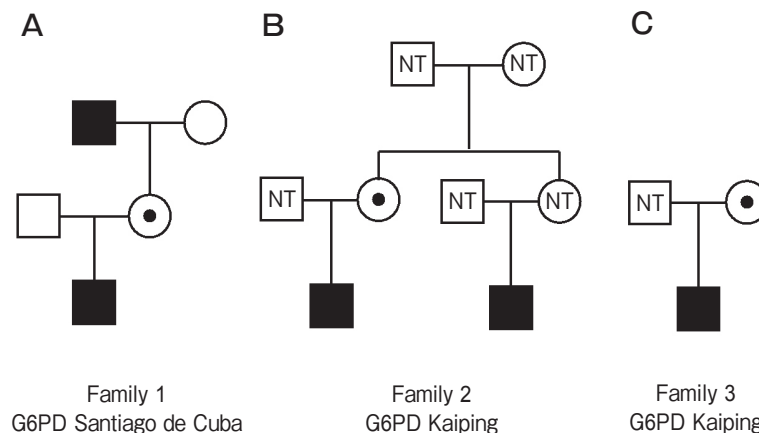


Fig. 1 Family trees of G6PD deficiency. ■, hemizygot of G6PD deficiency; ⊙, heterozygot of G6PD deficiency; □ and ○, full activity of G6PD; NT, not tested. **A**, Family 1 was found in Liaoning Province in northeastern China. **B** and **C**, Families 2 & 3 were from Hubei Province in central China.

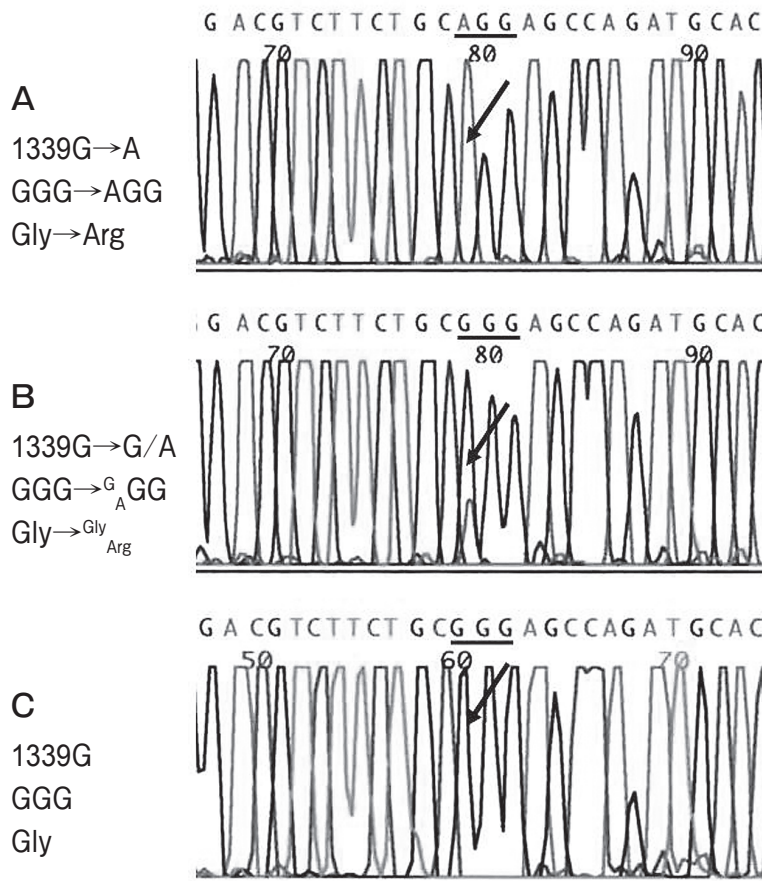


Fig. 2 **A**, Part of the DNA sequence in exon 11 from a hemizygous boy with G6PD Santiago de Cuba; **B**, Part of the DNA sequence in exon 11 from a heterozygous mother with G6PD Santiago de Cuba; **C**, Part of the DNA sequence in exon 11 from the boy's father with normal G6PD. Arrows show 1339A, 1339G/A, and 1339G.

variant in the Chinese population.

Discussion

Mutation 1339 G>A was first discovered in a Cuban boy, who showed heavy chronic anemia, and was therefore designated G6PD Santiago de Cuba [30]. Because the G6PD activity of this variant is very low, patients with this mutation show chronic hemolysis and anemia. Thus this variant is categorized as class I. Since the amino-acid substitution occurs at site 447, which is close to the dimer interface site [3], the variant molecule might not be formed properly, causing the enzyme activity to decline.

In the literature, 28 types of G6PD variant have

been reported in the Chinese population [19–29], and all are categorized as class II or class III, because they do not show chronic hemolytic anemia. In such patients, accidental hemolysis occurs only when they ingest certain oxidative medicines or foods. The present case and his family members with the G6PD Santiago de Cuba variant are thus the first reported cases of class I G6PD deficiency in a Chinese population.

In addition to Cuba and China, the G6PD Santiago de Cuba variant has also been reported in Japan [31]. The patient lived in Iwate Prefecture in northern Japan, and also suffered from chronic hemolysis and anemia. This is the only known case of G6PD Santiago de Cuba in the Japanese population. Fiorelli *et al.* reported that G6PD variants causing chronic hemolytic anemia are all sporadic, and almost all arise from independent mutations [32]. Moreover, because this variant, G6PD Santiago de Cuba, has been reported in Cuba, Japan and China, and the number of cases is very few, it is not likely to spread by genetic drift. This suggests that its origin is unlikely to be a common ancestor and that it is probably a new mutation that has arisen independently.

Among the blood samples from 414 males and 105 females collected in Liaoning Province in northeastern China, there were no cases of G6PD deficiency. This result suggests that the frequency of G6PD deficiency is very low in this area. Similar results have been reported by Jiang *et al.*, who found that the prevalence of G6PD deficiency was 0% (0/1,000) in Shandong Province in northern China [21]. Luzzatto *et al.* reported that the frequency of G6PD deficiency was high in malaria-endemic areas [33]. Individuals with G6PD deficiency are relatively resistant to falciparum malaria, and thus there is natural selection for G6PD-deficient people in areas with malignant malaria [34]. As a result, the frequency of G6PD deficiency is higher in malaria-endemic areas than in areas where

falciparum malaria has not existed. In northern areas of China, no falciparum malaria has existed historically. This is likely the reason that we found no G6PD-deficient cases but sporadic individual in this area.

Falciparum malaria was endemic to Hubei Province in the 1950s to 1970s [35], which is probably why the frequency of G6PD deficiency was high (7.4%) in our very small sampling of this area. Although we could not collect more samples from this area, this rate was similar to that from the southern part of China. Studies of G6PD deficiency in China have been done mainly in the southern part of the country. A high frequency of G6PD deficiency (5.2%) has been reported in Yunnan Province, the Guangxi Zhuang Autonomous Region, Guandong Province and Fujian Province [21]. Our results suggested that the distribution of G6PD deficiency in the northern regions of China was different from that in the central and southern areas. Based only on our findings, we might speculate that the frequency of G6PD deficiency and falciparum malaria are positively correlated in China.

In the Chinese population, the most common variants of G6PD are G6PD Kaiping (1388G > A), G6PD Canton (1376G > T), G6PD Gaohe (95G > A), G6PD Quing Yuan (392C > T) and G6PD Chinese-5 (1024C > T) [36]. These variants are frequently detected in Malaysia [37], Indonesia [10] and Vietnam [11], suggesting that they spread from China to these countries. Historically, Chinese people have migrated and settled in many countries and geographical regions. When G6PD variants in mainland China are compared with those in other areas, we can detect gene flows between Chinese and other ethnicities. For instance, G6PD Mediterranean (563C > T), which is common in Mediterranean countries, is detected in the Chinese population living in Singapore [27], but this variant is never seen on the mainland of China. We previously found G6PD Chatham in a Chinese individual living in Surabaya, Indonesia [5]. This variant has not been reported in mainland China, but is common in Indonesia [10] and Iran [38].

In conclusion, a patient with G6PD deficiency was found in northeastern China. This patient and his family members had a mutation of G6PD Santiago de Cuba, which was classified as class I according to its very low enzyme activity. This is the first reported

case of a class I G6PD deficiency in the Chinese population. In addition, our screening results suggest that the difference in the distribution of G6PD deficiency in China may be related to the endemicity of falciparum malaria.

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