Clin. Lab. 2024;70:XXX-XXX

CASE REPORT

An Unexpected Finding of Klinefelter Syndrome during Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD) Genetic Analysis

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SUMMARY

Background: **Klinefelter syndrome is a common sex chromosome abnormality in males, characterized by an extra X chromosome compared to normal males. Glucose-6-phosphate dehydrogenase deficiency (G6PD) is an X-linked incomplete dominant defect disorder. In this study, we reported the unexpected detection of Klinefelter syndrome in a patient with G6PD.**
Methods: **G6PD enzyme activity was measured by immunoenzyme assay, and genetic analysis was performed using a fluorescent PCR melting curve method (PCR-melting curve). Sex chromosome number abnormalities were detected by multiplex ligation-dependent probe amplification (MLPA). The patient also underwent peripheral blood chromosome karyotype analysis.**
Results: **The patient's G6PD and 6PGD enzyme activities were 21.34 U/L and 22.85 U/L, respectively, and their ratio was below the reference range (0.93). The PCR-melting curve displayed a c.1388 heterozygous mutation in this boy, and the Sanger sequencing provided the same results. MLPA results suggested the presence of approximately two copies of the X-chromosome in the boy. Finally, chromosome karyotype analysis confirmed that the boy had Klinefelter syndrome with a karyotype of 47, XXY.**
Conclusions: **Klinefelter syndrome was accidentally detected during G6PD genetic analysis in a male. X-chromosomes can interfere with the results of G6PD genetic analysis and should be noted.**
(Clin. Lab. 2024;70:xx-xx. DOI: 10.7754/Clin.Lab.2024.240105)

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KEYWORDS

glucose-6-phospate dehydrogenase deficiency (G6PD), Klinefelter syndrome, 47, XXY, enzyme activity

Case Report accepted January 4, 2024

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common X-linked genetic disorder, and about 400 million people are affected in the world [1]. The incidence rate of G6PD in Chinese neonates was 0.767%, with the highest rate of 3.387% in Guangxi [2]. G6PD-deficient people are susceptible to acute hemolytic anemia and severe jaundice during the neonatal stage when they consume particular foods, such as fava beans, or are exposed to certain diseases and medications [3].

G6PD deficiency is relevant to newborns because newborns at risk for G6PD face a deficit in developing non-physiologic hyperbilirubinemia. An increased serum bilirubin level (SBR) can cross the blood-brain barrier and result in a range of neurological disorders, including acute bilirubin-induced encephalopathy, kernicterus (chronic neurological disease), and even death [3]. In 1989, the WHO recommended newborn screening in regions where G6PD deficiency is prevalent [4]. Nowadays, newborn screening for G6PD enzyme activity has become a routine test in Chinese hospitals.

Klinefelter syndrome is the most common sex-chromosome disorder and is found in approximately 1 in 660 men [5]. The characteristic of this syndrome is the presence of one or more additional X chromosomes, with karyotype 47, XXY being the most prevalent type. Due to the great variation in clinical manifestations and the lack of professional awareness of the syndrome itself, only 25% of patients are diagnosed, and only a few of these are diagnosed before puberty, with many cases remaining undiagnosed [6]. Early recognition and hormonal treatment of the disease can ensure the development of appropriate masculinization of sexual characteristics, muscle size, and bone structure and prevent the long-term harmful consequences of hypogonadism [7]. Therefore, accurate diagnosis of the Klinefelter syndrome and the implementation of intervention measures are crucial. In this study, we demonstrate the unexpected diagnosis of the Klinefelter syndrome during G6PD genetic analysis.

CASE REPORT

A 2-year-old boy was referred to our hospital with a recurrent fever for five days and a cough for one day. He had no relevant medical or surgical history. A medical examination was made for this patient, and no abnormalities were found. His complete blood count (CBC) revealed white blood cell 5.98 x 10^9/L (reference: 4 - 10 x 10^9/L), hemoglobin 132.00 g/L (reference: 110.00 - 140.00 g/L), percentage of neutrophil 50.50% (reference: 50 - 75%), percentage of lymphocytes (reference: 20 - 40%), and platelet 66.00 x 10^9/L (reference: 100 - 300 x 10^9/L) (Sysmex XN 2800; Sysmex Corporation, Kobe, Japan). Other abnormal laboratory test findings included serum iron 3.31 μmol/L (reference: 11 - 30 μmol/L), high-sensitivity C-reactive protein (RP) > 5.0 mg/L (reference: 0 - 3 mg/L), lactate dehydrogenase (LDH) 414.0 U/L (reference: U/L), hydroxybutyric dehydrogenase (HBDH) 350.0 U/L (reference: U/L), creatine kinase MB isoenzymes (CK-MB) 47.1 U/L (reference: U/L), and G6PD/6PGD enzyme activity ratio 0.93 (reference: 1.0 - 2.3) (Cobas 8000; Roche Diagnostic, Basel, Switzerland).

Based on the G6PD enzyme activity ratio, the genetic analysis was performed on SLAN-96S (Hongshi Biotech, Shanghai, China) by a fluorescence PCR melting curve (PCR-melting curve). The kit for G6PD gene diagnosis can detect 12 mutations common in the Chinese population (Zhishan Biotech, Xiamen, China). The test results suggested a double peak in the Cy5 channel of reaction system A, and the Tm was 4.34℃ (67.5℃ - 63.16℃), corresponding to the mutation site c.1388 G>A (Figure 1A). The patient was male, but the mutation was indeed heterozygous, and we had to double-check with Sanger sequencing. As expected, the Sanger sequencing results also suggested a heterozygous mutation (Figure 1B). This indicated that the patient may have two X chromosomes. We then started by screening the sex chromosome with multiplex ligation-dependent probe amplification (MLPA). The SALSA MLPA P095 kit aims to recognize the potential etiology and clinical diagnosis of Patau, Edwards, Down Syndrome (Trisomy 13, 18, and 21, respectively), Turner, Triple X, Klinefelter, 47, XXY syndromes (X/Y chromosome aneuploidy) (MRC-Holland, Amsterdam, The Netherlands). Meanwhile, the patient was advised to recollect peripheral blood for chromosome karyotype analysis. The MLPA results showed that the Y-chromosome probe had a probe ratio in the range of normal males and that the X-chromosome probe was approximately two times that of normal males, which was suspected to be Klinefelter syndrome (karyotype 47, XXY). Chromosome karyotype analysis confirmed that the patient's karyotype was 47, XXY.

DISCUSSION

Variants of the G6PD gene lead to deficiency in protein and activity, impairing the regulation of the redox state. Females carry two copies of the G6PD gene and have three phenotypes: homozygous for normal alleles (g6pd norm/norm), heterozygous mutation (g6pd def/norm), and homozygous mutation (g6pd def/def) [3]. G6PD enzyme activity varies among the different types, with wild-type homozygous females generally having greater than 80% of normal activity levels, homozygous mutation activity less than 30%, and heterozygous mutation enzyme activity being broader, ranging from 20 - 80% [3]. For males with only one copy of the G6PD gene, G6PD activity levels are either normal or defective. In this present study, the boy had a slightly lower percentage of G6PD enzyme activity ratios, which cannot be excluded due to measurement error. However, it was because of this small altered ratio that a genetic analysis was carried out, and the patient was accidentally diagnosed with Klinefelter syndrome.

The PCR-melting curve revealed that the Cy5 channel amplified two peaks in this boy, corresponding to the c.1388 G>A heterozygous mutation. As we all know, males carry only one copy of the G6PD gene, and if a mutation occurs, it should be a homozygous mutation peak (single peak) on the PCR peak graph. This showed that the G6PD test results of the boy contradicted the theory. We had to perform a double-check to rule out the effects of sample error, but the result was still a he-

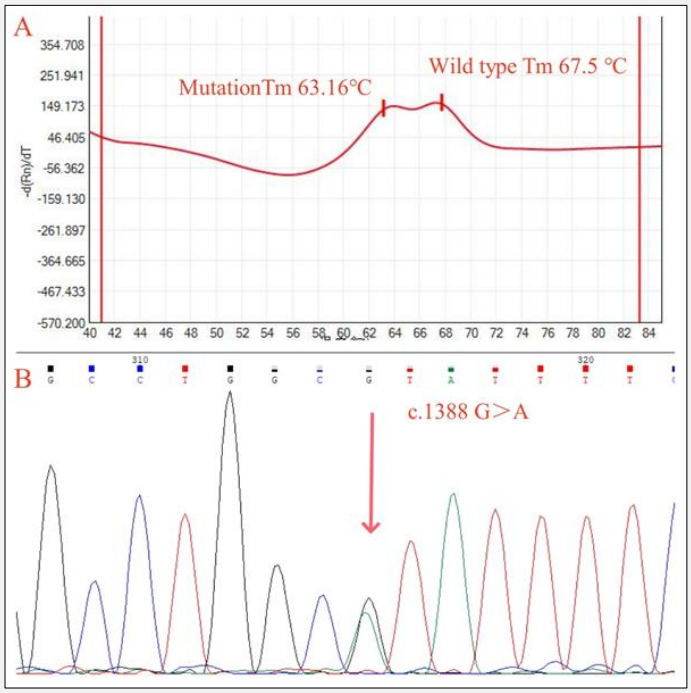

Figure 1. PCR-melting curve (A) and Sanger sequencing (B) results for this patient.

Both methods showed that c.1388G>A were heterozygous in the G6PD gene.

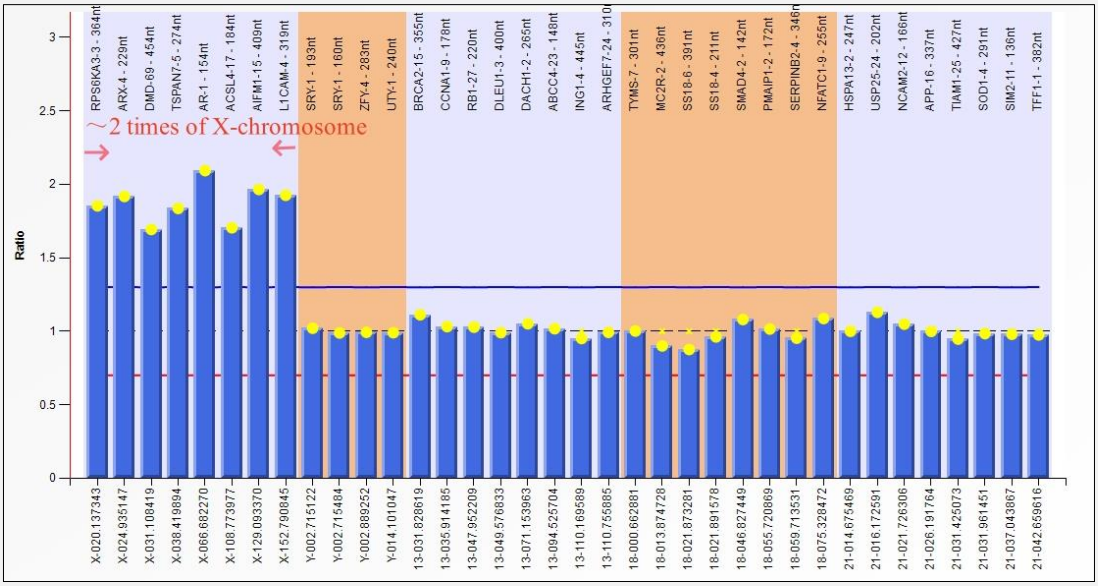

Figure 2. MLPA showed about two copies of the X chromosome in the patient.

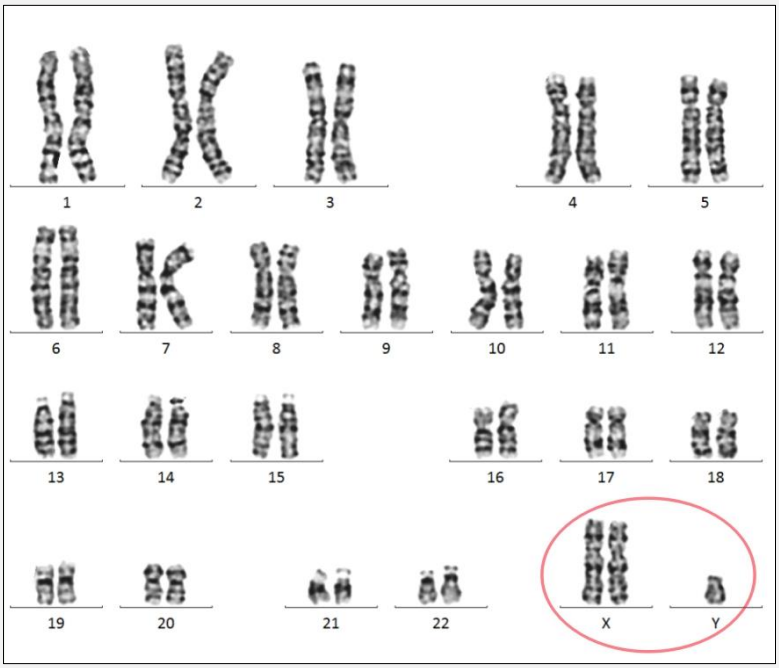

Figure 3. Chromosome karyotype analysis confirmed that the boy had Klinefelter syndrome with a karyotype of 47, XXY.

terozygous mutation. Subsequently, we validated using Sanger sequencing, and it again was a c.1388 G>A heterozygous mutation. These suggested that the boy may have an extra X chromosome compared to normal males. In further research, MLPA was used to screen for abnormal numbers of sex chromosomes. About twice the number of copies of the normal X chromosome was detected in this boy; according to the manufacturer's instructions, he could be diagnosed as 47, XXY. At the cellular level, chromosomal karyotyping confirmed the diagnosis of Klinefelter syndrome in this boy, and no mosaic chromosomes were seen to be present.

As Klinefelter syndromeis an X-linked disease, it is more common in males than females. A range of clinical features have been described, such as tall stature, gynecomastia, small penis, cryptorchidism, azoospermia, cognitive impairment, and androgen deficiency [8, 9]. Undergoing hormone therapy can improve quality of life. In recent years, with the development of assisted reproductive technologies, the opportunity to have children has been offered to many patients with Klinefelter syndrome. Even in the absence of sperm in the semen, pregnancy and live birth can still be achieved by being able to extract sperm from a testicular biopsy sample [7, 10]. Most opportunities are lost owing to misdiagnosis, despite our growing understanding of the signs of Klinefelter syndrome, helpful therapies, and when to intervene. Therefore, the key to achieving early intervention is early diagnosis. In this study, the 2-year-old child had not yet demonstrated significant clinical features, but we rapidly diagnosed the Klinefelter syndrome by genetic analysis, saving time for early intervention.

CONCLUSION

In this case, we report the unexpected detection of the Klinefelter syndrome in a boy's G6PD genetic analysis. X chromosomes may cause misleading G6PD genetic analysis and affect the interpretation of the results in males. When there is an inconsistency between the results of the genetic analysis and the theory or pattern of inheritance, it is recommended that further MLPA or chromosomal karyotyping should be performed.

Declaration of Interest:

The authors report no conflicts of interest relevant to this article.

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