Clin. Lab. 2024;70:XXX-XXX

CASE REPORT

An Unexpected Detection of the Rare 48,XXYY in the Prenatal Diagnosis of a Fetus with β-Thalassemia Major

Wei Chen [1, *], Shaowen Ban [1, *], Zhaoying Zhu [1], Jie Chen [1], Yongxiong Yu [1], Jinping Bai [2], Youqiong Li [2]

[*] *These authors contributed equally to this work and should be considered co-first authors*
[1] *Department of Clinical Genetic Laboratory, Maternal and Child Health Care Hospital of Wuzhou, Wuzhou, Guangxi, China*
[2] *Center for Medical Genetics and Prenatal Diagnosis, People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi, China*

SUMMARY

Background: **Thalassemia is a common monogenic disorder, and children with β-thalassemia major require regular blood transfusions and iron removal therapy. Klinefelter syndrome (KS) is a common sex chromosome abnormality, and 48,XXYY is rare. This report is the first to describe a fetus with a karyotype of 48,XXYY in prenatal diagnosis of β-thalassemia major.**
Methods: **Amniotic fluid was collected by puncture for the prenatal diagnosis of thalassemia, and chromosomal karyotyping was also performed. PCR and reverse dot-blot hybridization (PCR-RDB) were used to identify 17 common β-thalassemia mutations in China. Karyotype analysis of amniotic fluid was performed.**
Results: **The results of PCR-RDB revealed that the genotype of the fetus was a homozygote of CDs41-42 (-TTCT) in the *HBB* gene. The karyotype analysis displayed that the fetus had Klinefelter syndrome (KS), and the karyotype was the rare 48,XXYY. The fetus was diagnosed with β-thalassemia major and KS.**
Conclusions: **An unexpected detection of the rare 48,XXYY in the prenatal diagnosis of a fetus with β-thalassemia major. There is a pitfall of genetic counseling and prenatal diagnosis in China.**
(Clin. Lab. 2024;70:xx-xx. DOI: 10.7754/Clin.Lab.2024.240517)

Correspondence:
Professor Youqiong Li
Center for Medical Genetics and
Prenatal Diagnosis
People's Hospital of
Guangxi Zhuang Autonomous Region
No. 6 Taoyuan Road
Nanning, Guangxi 530021
China
Email: liyouqiong327@163.com

KEYWORDS

thalassemia, Klinefelter syndrome, 48,XXYY, prenatal diagnosis

INTRODUCTION

Thalassemia is one of the most common monogenic disorders in the world due to defective production of the normal globin chain [1,2]. They are classified into α, β, δβ, and δβγ-thalassemia based on the specific globin chain synthesis that is impaired [3]. In clinical practice, α- and β-thalassemia are the most common. The most severe form of α-thalassemia is Hb Bart's hydrops fetalis, which often results in abortion during the second trimester or third trimester. Patients with β-thalassemia major require lifelong blood transfusions and iron chelator administration; otherwise, death occurs in the first few years of life [4]. Alternatively, β-thalassemia major

Case Report accepted May 21, 2024

is treated with bone marrow transplantation or gene therapy but faces difficulties in matching or technical limitations, as well as a high financial burden on the family [5,6]. Accurate diagnosis is important for genetic counseling and prenatal diagnosis of different types of thalassemia.

Klinefelter syndrome (KS) is the most common sex chromosome aneuploidy (SCA) in males, characterized by the presence of one or more extra X chromosomes [7]. Eighty to ninety percent of KS is non-mosaic karyotype (47, XXY), with the remaining 10 - 20% being mosaic karyotypes (e.g., 47, XXY/46, XY), higher-grade aneuploidies (e.g., 48,XXYY or 48,XXXY), or structurally abnormal X chromosomes (e.g., 47, iXq, Y) [8]. The clinical features of KS range from normal masculinization with only minor physical abnormalities to severe symptoms of androgen deficiency. The classical phenotype is that of gynecomastia, small penis, tiny testicles, high stature, azoospermia, and infertility. People only seek medical attention when they experience a range of typical symptoms. As a result, only 25% of individuals are diagnosed, and many are still not diagnosed nor effectively treated [9]. In this study, we accidentally detected 48,XXYY in a prenatal diagnosis from a fetus with β-thalassemia major.

CASE REPORT

A couple was referred to our hospital for a routine prenatal examination. Previously, when they came to our hospital for their first-trimester screening, they had both been diagnosed as carriers of β-thalassemia ($\beta^{CD41-42M}/\beta^{N}$) by genetic analysis. This time she was to follow the medical advice for the prenatal diagnosis of thalassemia. The female was 17 weeks pregnant, and her B-ultrasound results showed no abnormalities in the fetus. During genetic counseling, the doctor advised simultaneous chromosomal karyotyping with prenatal testing for thalassemia. The couple signed a consent form after giving informed consent. The amniotic fluid of the pregnant female was collected by puncture, mediated by B-ultrasound. The amniotic fluid was visually assessed for the absence of blood, turbidity, and other abnormalities. PCR-RDB is relatively sensitive and is prone to misdiagnosis if maternal cell contamination (MCC) is not excluded. Our hospital lacked a technique for identifying MCC, so we minimized it through amniotic fluid culture. After analyzing the cultured amniotic fluid cells, it was found that the fetus had β-thalassemia major (Figure 1) due to being homozygous for CDs41-42 in the HBB gene. A week later, chromosomal karyotyping was completed and unfortunately, the fetus was found to have a rare type of Klinefelter syndrome, 48,XXYY (Figure 2).

DISCUSSION

48,XXYY is a rare variant caused by an extra copy of both sex chromosomes. It is not inherited, and the variant occurs in 1 per 18,000 to 1 per 40,000 of all male births [10]. 48,XXYY syndrome has a similar phenotype to the other Klinefelter syndromes but is associated with a higher risk of neurocognitive disorders and attention deficit hyperactivity disorder [11]. In this present study, we described the unexpected detection of 48,XXYY during the prenatal diagnosis of a fetus with β-thalassemia major. To the best of our knowledge, this is the first report of a prenatal diagnosis of β-thalassemia major combined with 48,XXYY syndrome.

Individuals with β-thalassemia major require regular blood transfusions and medication to remove iron, resulting in a heavy financial burden on the family and unhealthy psychological growth for the children. The commonly used clinical test is PCR and reversed dot blot hybridization (PCR-RDB) in China, which is easy to perform and interpret the results, suitable for peripheral blood, chorionic villi, and amniotic fluid. In addition to hematopoietic stem cell transplantation, successful gene therapy has recently been reported for treating β-thalassemia major [12]. Gene therapy may be a complementary option in cases where hematopoietic stem cell donors and matching are difficult. Whether through hematopoietic stem cell therapy or gene therapy, early intervention and treatment tend to yield better outcomes. Therefore, prompt and precise screening and diagnosis of thalassemia allow for early intervention or the development of a treatment plan. By testing the cultured amniotic fluid, we accurately identified β-thalassemia major. This case provides an important alert that there is a pitfall in genetic counseling and prenatal diagnosis of thalassemia. The clinical pathway in Chinese hospitals was that when couples were at risk of having a child with severe thalassemia and no abnormalities were found on B-ultrasound, non-invasive DNA testing (NIPT), or maternal serological screening, prenatal diagnosis was performed for thalassemia only. However, it is possible for a fetus to have chromosomal abnormalities even if it has not inherited thalassemia mutations from its parents. In this study, the pregnant female did not follow her doctor's advice to screen for chromosomal disorders, either by maternal serological test or NIPT. Otherwise, the fetus with a sex chromosome abnormality might have been observed in the first trimester because of the high sensitivity of the NIPT. Fortunately, this fetus was accurately diagnosed with β-thalassemia major and 48,XXYY.

Based on this pitfall, we proposed that prenatal diagnosis of thalassemia or other monogenic inherited diseases should be accompanied by chromosomal karyotyping. This workflow is shown in Figure 3. The genetic analysis has been completed, and the karyotype analysis results will not be available for another month. If the results of the genetic analysis are severe thalassemia, the pregnant woman can be informed beforehand, regard-

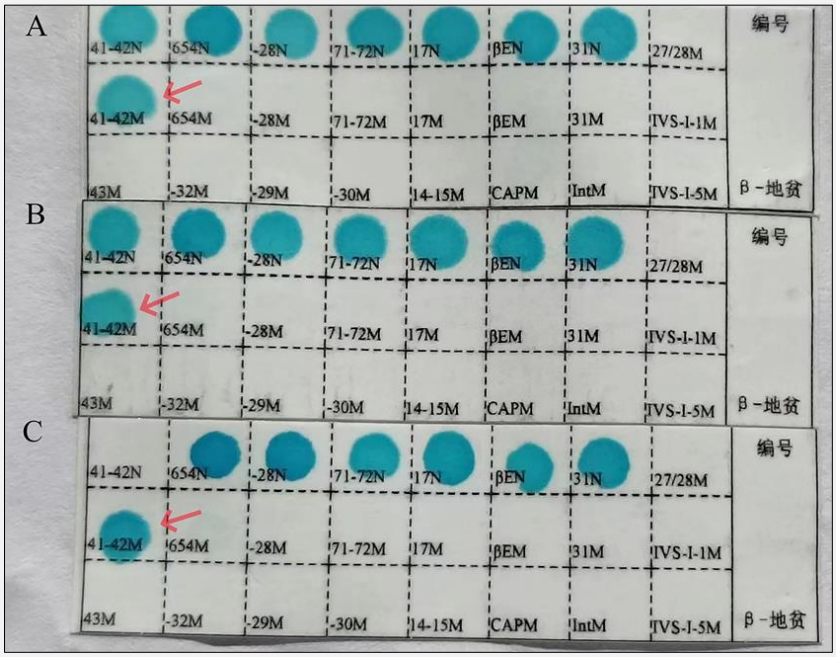

Figure 1. PCR-RDB results for the family.

Both male and female showed heterozygous mutations for CDs41-42 in the *HBB* gene, and the fetus presented homozygous mutations for CDs41-42.

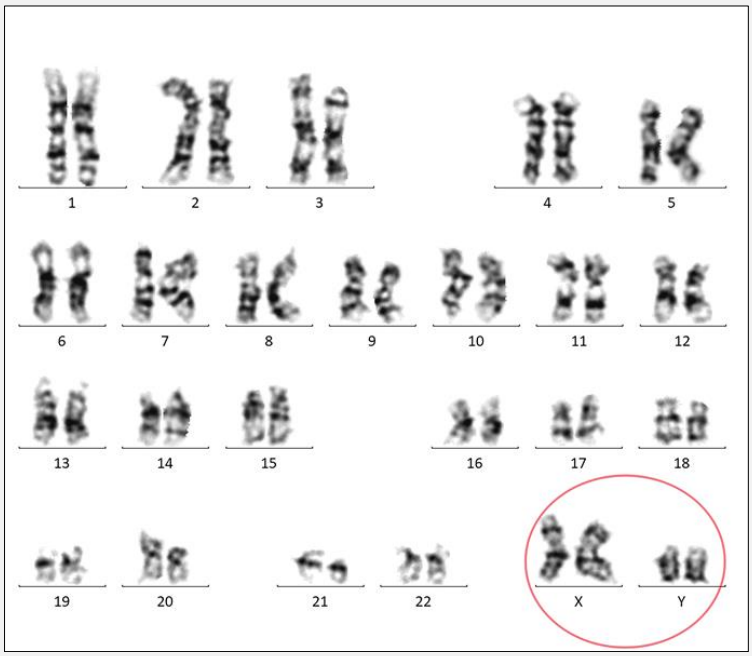

Figure 2. Chromosomal analysis of the fetus revealed a 48,XXYY karyotype.

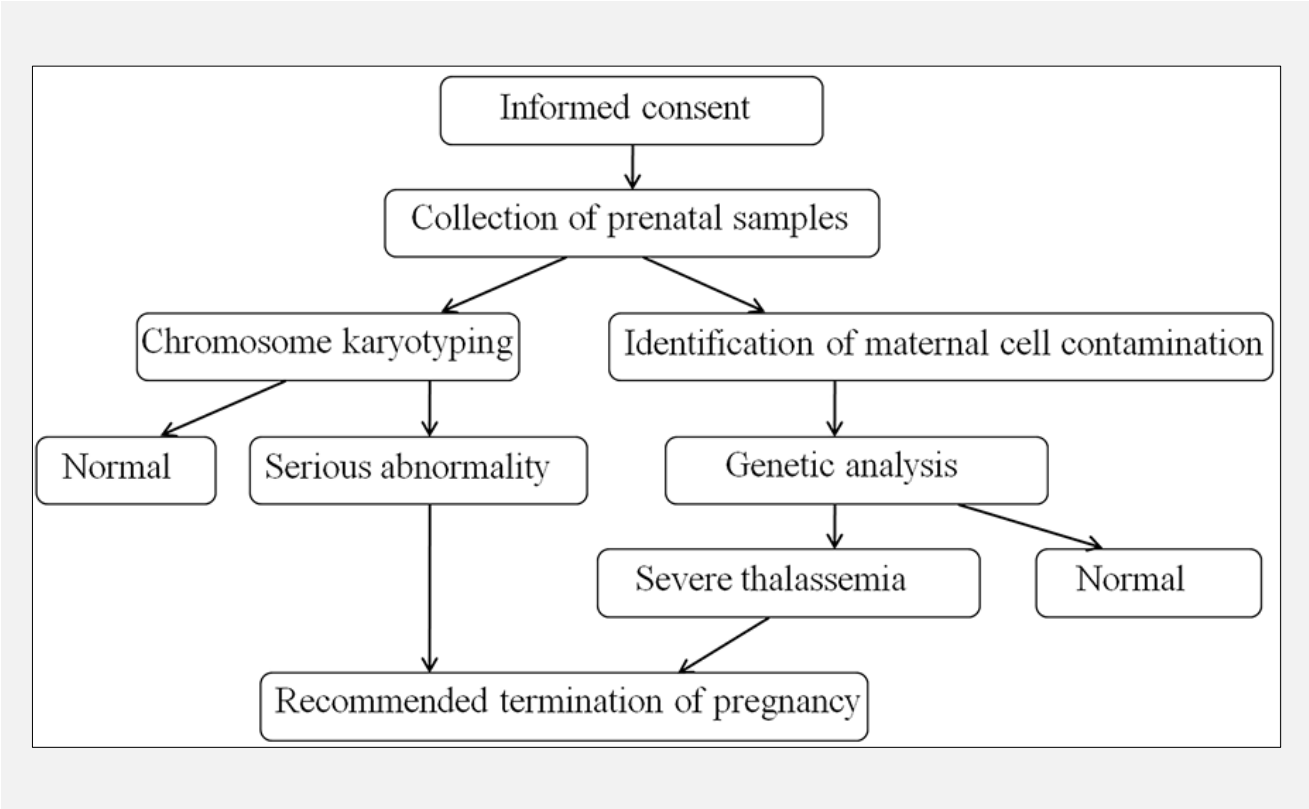

Figure 3. Recommended workflow for simultaneous thalassemia genetic analysis and chromosomal karyotyping.

less of the results of the karyotype analysis. In a case where the result of the genetic analysis is not severe thalassemia, the pregnant female will have to wait for the report of the chromosomal karyotype. Misdiagnosis of severe thalassemia and chromosomal disorders will be avoided by completing and optimizing the testing program.

CONCLUSION

This report was the first to describe a fetus with a karyotype of 48,XXYY in prenatal diagnosis of β-thalassemia major. This case demonstrates the pitfalls of prenatal diagnosis and genetic counseling for pregnant females at risk of having a child with severe thalassemia in China. During prenatal diagnosis of thalassemia, chromosomal karyotyping is proposed to be performed simultaneously to avoid possible chromosomal abnormalities in cases where the genetic analysis for thalassemia is normal.

Source of Funds:
This study was supported by the Scientific Research of Wuzhou Health Commission, Guangxi (Grant Number WZWS-F2023021) and the Natural Science Foundation of Guangxi (Grant Number 2023GXNSFAA026102).

Declaration of Interest:
No potential conflict of interest was reported by the author(s).

References:

1. Lee JS, Cho SI, Park SS, Seong MW. Molecular basis and diagnosis of thalassemia. Blood Res 2021;56(S1):S39-S43. (PMID: 33935034)

2. Mettananda S, Higgs DR. Molecular Basis and Genetic Modifiers of Thalassemia. Hematol Oncol Clin North Am 2018;32(2):177-91. (PMID: 29458725)

3. Munkongdee T, Chen P, Winichagoon P, Fucharoen S, Paiboonsukwong K. Update in Laboratory Diagnosis of Thalassemia. Front Mol Biosci 2020;7:74. (PMID: 32671092)

4. Kwiatkowski JL. Gene addition for beta thalassemia. Ann N Y Acad Sci 2023;1530(1):105-9. (PMID: 37828865)

5. Christakopoulos GE, Telange R, Yen J, Weiss MJ. Gene Therapy and Gene Editing for β-Thalassemia. Hematol Oncol Clin North Am 2023;37(2):433-47. (PMID: 36907613)

6. Yousuf R, Akter S, Wasek SM, Sinha S, Ahmad R, Haque M. Thalassemia: A Review of the Challenges to the Families and Caregivers. Cureus 2022;14(12):e32491. (PMID: 36523854)

7. Sa R, Ferraz L, Barros A, Sousa M. The Klinefelter Syndrome and Testicular Sperm Retrieval Outcomes. Genes (Basel) 2023;14 (3):647. (PMID: 36980920)

8. Bonomi M, Rochira V, Pasquali D, et al. Klinefelter syndrome (KS): genetics, clinical phenotype and hypogonadism. J Endocrinol Invest 2017;40(2):123-34. (PMID: 27644703)

9. White M, Zacharin MR, Fawcett S, et al. Klinefelter Syndrome: What should we tell prospective parents? Prenat Diagn 2023;43 (2):240-9. (PMID: 36225116)

10. Razali NA, Mohd Daud TI, Woon LS, Mohamed Saini S, Muhammad NA, Sharip S. Case report: Bipolar disorder in 48, XXYY syndrome. Front Psychiatry 2023;13:1080698. (PMID: 36713919)

11. Pai LF, Wang DS, Chu DM. A rare case of 48, XXYY syndrome in Taiwan. Pediatr Neonatol. 2022;63(5):559-60. (PMID: 35659752)

12. Wang LR, Li LX, Ma YL, et al. Reactivation of γ-globin expression through Cas9 or base editor to treat β-hemoglobinopathies. Cell Res 2020;30(3):2768. (PMID: 31911671)