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CASE REPORT

Combined Application of Multiple Techniques in Prenatal Diagnosis of a Fetus with Turner Syndrome

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SUMMARY

Background: **The clinical features of Turner syndrome (TS) involve multiple organ system dysplasia, among which growth retardation and gonadal dysplasia are the most important clinical phenotypes.**
Methods: **G banding karyotype analysis, chromosome microarray (CMA), and fluorescence in situ hybridization (FISH) were used for prenatal diagnosis of fetal chromosomes.**
Results: **The result of fetal chromosome karyotype analysis was 46,XX. CMA showed arr[GRCh38]Xp22.33 p22.13(251888_18176046)x1,Xq27.1q28(140998347_156003433)x3. FISH indicated that the short arm end fragment of X chromosome was monomer and the long arm end fragment was trisomy.**
Conclusions: **The fetal chromosome karyotype was normal, but CMA indicated that there was deletion and duplication of X chromosome. FISH verified the CMA results, locating the deletion and duplication fragments. CMA and FISH make up for the shortcomings of chromosome karyotype analysis technique. It is suggested that multiple detection methods should be applied in genetic prenatal diagnosis.**
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KEYWORDS

chromosome, karyotype, CMA, FISH

INTRODUCTION

TS known as congenital ovarian hypoplasia is a rare female sex chromosome disease caused by the complete or partial deletion of an X chromosome in all or part of somatic cells or other structural abnormalities of the X chromosome. The incidence rate is about 1/2,500 - 1/2,000 of newborn girls. The clinical manifestations are mainly short stature and gonadal dysplasia, which may be accompanied by webbed neck, lymphedema, skeletal dysplasia, congenital cardiovascular malformation and so on, but the degree of intellectual development varies [1,2]. In recent years, the detection rate of fetal chromosomal karyotype has increased with the widespread application of prenatal diagnosis cytogenetics, resulting in a decreasing incidence of neonatal TS. However, the small abnormal segment of X chromosome, which is not easily detected by karyotype technique, is easily

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missed in prenatal diagnosis. In this study, three detection techniques were used to identify the structural abnormality of one X chromosome, which clarified the importance of the combined application of multiple techniques in prenatal diagnosis.

SUBJECT AND METHODS

Clinical case

The patient was female, 42 years old, who had given birth to one healthy boy in 2002. At the 19th week of this pregnancy, she came to Shaoxing Maternity and Child Health Care Hospital on February 23, 2023, for amniotic cavity puncture because of old age. The amniotic fluid was sent for chromosome G banding karyotype analysis, CMA and FISH. The phenotype and intelligence of the patient and her husband were normal. Their chromosome karyotypes in peripheral blood were normal, too.

Research methods

G banding karyotype analysis: About 16 mL of amniotic fluid was extracted and cultured independently in two lines using pancreatic enzyme digestion method. The karyotype of the amniotic fluid was described according to International System for Human Cytogenetic Nomenclature (ISCN2020).

CMA: About 10 mL amniotic fluid was extracted for DNA extraction, enzyme digestion, ligation, PCR, PCR product purification, fragmentation, labeling, hybridization, and other steps. ChAS4.0 was used for the analysis, and for the result interpretation we referred to DGV, DECIPHER, PubMed, OMIM, ClinGen databases and related literature reports.

FISH: The medium chromosome slides were prepared using the G banding residual cell suspension. The probe combination solution for the end of the short arm of XY chromosomes (green signal), the end of the long arm (red signal), and the centromere region (white signal) was prepared according to the instructions (Abbott-Vysis, USA). The slides and probes were hybridized, washed, and re-dyed. Then the slides were observed and analyzed under fluorescence microscope. FISH 2.1 software was used to photograph 20 mesomeric phases under 100 x oil lens with a fluorescence microscope. Determining the deletion or duplication of X and Y chromosome ends was based on the centromere signal of X chromosome, the green signal at the ends of short arms and the red signal at the ends of long arms.

RESULTS

The fetal chromosome karyotype was 46,XX. The results of CMA were arr[GRCh38]Xp22.33p22.13(251888_18176046)x1,Xq27.1q28(140998347_156003433)x3 (Table 1). FISH signals were normal in the samples of the patient and her husband. In the amniotic fluid sample, two red signals and no green signals were observed on der(X), indicating that the monomer of the short arm end fragment combined with the trisomy of the long arm end fragment of X chromosome in amniotic fluid cells (Figure 1). The fetus was diagnosed as a new variant in their family. After genetic counseling and informed consent of the family, the patient terminated the pregnancy at 22 weeks of gestation and refused further related tests on the fetus.

DISCUSSION

The chromosome karyotype of amniotic fluid cells was normal, but CMA indicated that the X chromosome had deletion and duplication. The deletion completely contained Leri-Weill dyschondrostosis (LWD) and Steroid sulphatase deficiency (STS). LWD is a nonfatal skeletal dysplasia, more than 60% of the cases are caused by heterozygosity loss and mutations of the short stature homeobox contain inggene (SHOX). The clinical phenotypes are diverse, mainly including disproportionate short stature, mid-limb dysplasia (short forearm and lower leg), Madelon's deformity, and other skeletal development abnormalities. Some patients may have conductive hearing loss, premature craniosynostosis, sciatic dysplasia, dental and maxillofacial abnormalities, and a few patients only show mild short stature [3-6]. SHOX is a growth control gene that participates in bone development and is located in the Xp22 region. Mutations or deletions in SHOX can lead to insufficient single dose, resulting in short stature and abnormal bone development [7]. The main clinical manifestation of STS is ichthyosis, which is usually X-linked recessive inheritance with male onset and female carriers. However, the possibility of onset in female due to X chromosome inactivation cannot be completely ruled out. The main clinical manifestations of Xq28 duplication are speech and language retardation, intellectual impairment, hypotonia, recurrent infection, epilepsy, spasm, and so on. FISH detected that the X long arm duplicate segment was just attached to the missing short arm segment, resulting in no visual abnormalities in chromosome morphology. In addition to G banding karyotype analysis, CMA and FISH were used to identify the regions, locations, and key genes of deleted and duplicate fragments, making up for the shortcomings of missing diagnosis by karyotype analysis alone. It can be seen that the combined application of multiple detection methods in genetic prenatal diagnosis is crucial, which can provide more accurate prenatal diagnosis and enrich the theory of prenatal genetic counseling for fetuses with chromosomal abnormalities.

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Table 1. CMA results and major OMIM genes.

Genomic locus	CNV type	Length	Chromosome abnormality judgment	Part of the OMIM gene
arr[GRCh38] Xp22.33p22.13(251888_18176046)x1	deletion	17,924 kb	pathogenicity	PLCXD1 (300974), GTPBP6 (300124), PPP2R3B (300339), SHOX (312865), SHOX (400020)
arr[GRCh38] Xq27.1q28(140998347_156003433)x3	duplication	15,005 kb	pathogenicity	SPANXB1 (300669), RNU6-1 (180692), LDOC1 (300402), SPANXC (300330), SPANXA1 (300305)

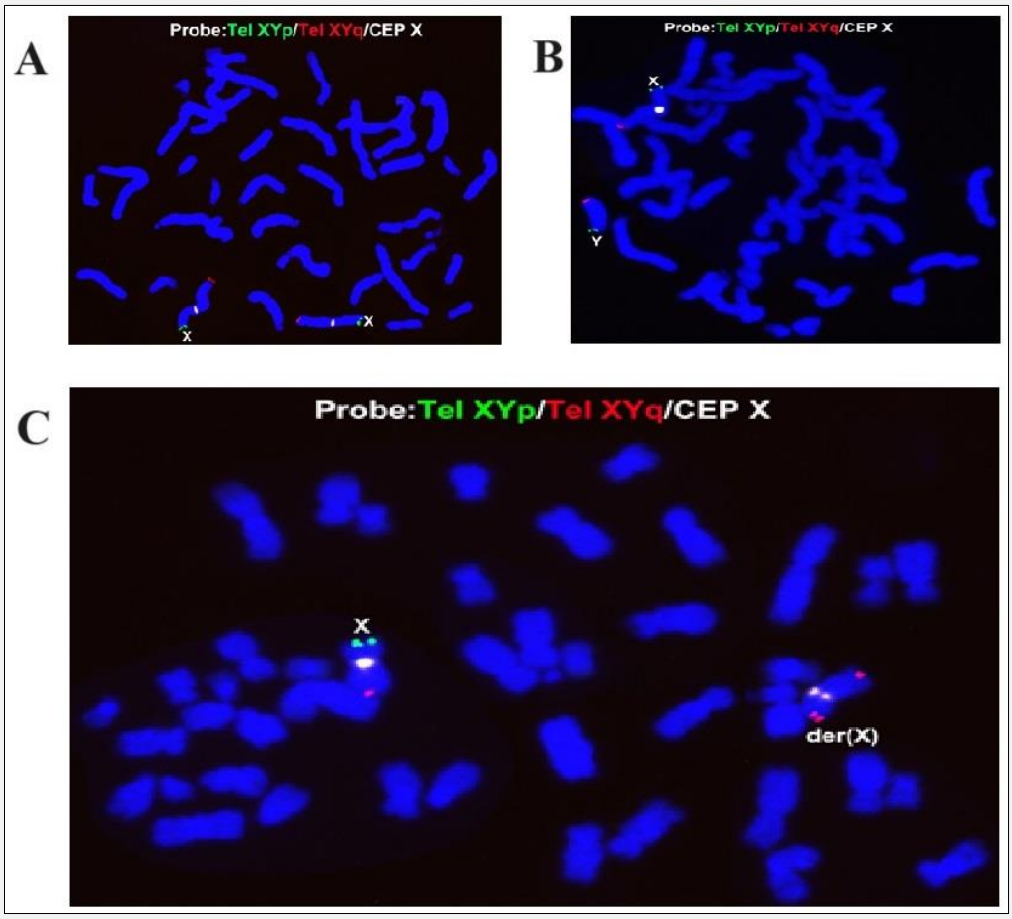

Figure 1. (A) The patient's FISH diagram. (B) Her husband's FISH diagram. (A) and (B) were normal. (C) The fetus' FISH diagram. The der(X) was abnormal. It had no short arm end fragment and had two long arm end fragments.

Declaration of Interest:
All authors declare that they have no competing interests.

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