CASE REPORT


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SUMMARY

Background: Amniocentesis was performed on a pregnant woman with a deletion of exon 45 of the Duchenne Muscular Dystrophy (DMD) gene.

Methods: Fetal Xp21.1 (31944831-32030363) x 0 was found by chromosome microarray analysis (CMA), i.e., 0.086 Mb hemizygote deletion was detected in the Xp21.1 region of the fetal X chromosome, which contained exon 45 of the DMD gene.

Results: The results verified by MLPA were consistent with those of CMA, which indicated that CMA was accurate in a single exon deletion in this fetus. This case suggests that CMA may become an essential method for the prenatal diagnosis of a fetus with DMD gene deletion/duplication.

Conclusions: It can routinely detect chromosome copy number variation and analyze DMD diseases caused by exon duplication or deletion, which is enormously significant for new DMD exon deletion or duplication. (Clin. Lab. 2022;68:xx-xx. DOI: 10.7754/Clin.Lab.2021.210750)

KEY WORDS

Chromosome microarray analysis, DMD, exon, prenatal diagnosis, MLPA

INTRODUCTION

Duchenne muscular dystrophy (DMD) is a severe, progressive, muscle-wasting disease that leads to difficulties with movement beginning around 2 - 3 years of age [1], to wheelchair dependence around 10 - 12 years of age, and eventually, to death, usually before the age of 30 [2]. DMD is caused by mutations in the DMD gene (encoding dystrophin, OMIM *300377) that occur in the short arm of the X chromosome (Xp21.1) and is one of the largest known human genes, covering 2.2 Mb and containing 79 exons [3]. With an X-linked recessive inheritance pattern, a mother with this mutation has a 50% chance of transmitting it to her male children, affecting approximately one in 3,500/5,000 liveborn boys [4]. Although most DMD mutations are inherited, spontaneous mutations can occur in up to 30% of cases [5]. About 60.2% of DMD/Becker muscular dystrophy (BMD) gene variation is caused by the deletion of one
or more large exons, 9.6% by large fragment duplications, and 23.3% by point mutation [3]. Multiplex ligation-dependent probe amplification (MLPA) is the main diagnostic method for exon deletion or duplication, and Sanger sequencing or second-generation sequencing is the main diagnostic method for point mutation. For pregnant women with a family history of DMD, prenatal diagnosis with MLPA or Sanger sequencing can be used to diagnose DMD in the fetus and reduce congenital disabilities. However, the spontaneous mutation rate of DMD is as high as 30%, and this part of the gene with spontaneous mutation is likely to be missed because there is no family history, and no special DMD genetic diagnostic tests have been arranged. We report a fetus with a single exon deletion of the DMD gene found during routine CMA, which suggests a novel approach to preventing missed diagnosis and delivery of babies with spontaneous mutation of the DMD gene.

CASE REPORT

A 32-year-old pregnant woman with loss of heterozygosity at exon 45 of the DMD gene already had one child with DMD. At the 18th week of this pregnancy, an ultrasound-guided amniocentesis was performed, and 35 mL amniotic fluid was aspirated and divided into three aliquots. One aliquot was used for cell culture for fetal chromosome karyotype analysis, and the second aliquot was used to extract DNA to detect chromosome microdeletion and microduplication by CMA. The third aliquot was used to detect deletion/duplication of 79 exons of fetal DMD gene by MLPA combined with capillary electrophoresis.

Genetic test results

No abnormality was found in the analysis of G banding 320-400 of the fetal chromosome. The fetal CMA revealed arr [grch38] Xp21.1 (31944831-32030363) x 0, i.e., 0.086 Mb hemizygote deletion was detected in xp 21.1 region of the fetal X chromosome (Figure 1 and Figure 2). The fetal DMD gene exon deletion analysis revealed that fetal exon 45 was deleted (Figure 3), i.e., the fetus was likely to be a DMD patient, and the patient opted to terminate the pregnancy. The Ethics Committee of the First Hospital of Jilin University, Changchun, Jilin, China, approved this research. This study was approved by the Ethics Committee of First Hospital of Jilin University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in this study.

DISCUSSION

We report a fetus with 0.086 Mb hemizygote deletion detected by CMA in the Xp21.1 region of the fetal X chromosome, which contained exon 45 of the DMD gene. The result was confirmed by MLPA detection technology. The fetal chromosome CMA was performed using the CytoScan 750 K array technology platform of Affymetrix company. The chip used in this technology platform contains more than 750,000 genetic marker probes, including 550,000 non-polymorphic probes and 200,000 Single Nucleotide Polymorphism Microarray (SNP) probes. There is one probe per 1 kb in ISCA (International Science Community Association) gene on average. For the copy number change of > 400 kb, the sensitivity and specificity were higher than 99%, but the accuracy of < 50 probe markers or < 100 kb copy number variation decreased. The average length of a single exon of the human gene is 145 bp and therefore it is challenging to diagnose a single exon deletion by CMA. Pseudohypertrophic muscular dystrophy [DMD (#310200)] is an X-linked recessive single-gene neuromuscular disease with high incidence and significant impairment, of which 60.2% is caused by deletion of one or more large exons, 9.6% by duplication of large fragments, and 23.3% by new mutations [3]. The DMD gene (*300377) is one of the largest known human genes, containing 79 exons and with a length of about 2.2 Mb [3]. With the increase of probes for the DMD gene region on the Cytoscan 750 K chip, deletion and duplication of a single exon of the DMD gene may be captured and diagnosed by CMA technology. In this case, the fetal DNA was analyzed with CMA technology, and fetal Xp21.1 (31944831-32030363) x 0 was detected, i.e., there was 0.086 Mb hemizygote deletion in the fetal X chromosome Xp21.1 region, which covered exon 45 of the DMD gene. The fetus was likely to have a deletion of exon 45 of the DMD gene. The MLPA also showed that the fetal exon 45 was deleted (Figure 3). The results were consistent, indicating that CMA was accurate in diagnosing fetal exon 45 deletions. Although only a minor variant of the X chromosome was found in the whole genome map by CMA (Figure 1), it was still possible, by analyzing the enlarged X chromosome image obtained by CMA (Figure 2), to locate the specific missing or duplicate probes, and thus judge the position, size, and constituent genes of the chromosome variant. Therefore, as long as technicians carefully analyze the results, CMA may become important for prenatal diagnosis of fetuses with DMD gene deletion/duplication. The DMD diseases caused by exon 45 duplication or deletion can be analyzed simultaneously as routine detection of chromosome copy number variation, essential for detecting new DMD exon deletion or duplication. If the probe density of the chip in this gene region can be increased, the accuracy and reliability may be further improved.
Figure 1. Fetal CMA genome map. The arrow shows the location of a small deletion on the X chromosome.

Figure 2. CMA map of the fetal X chromosome suggests Xp21.1(31944831-32030363) x 0.
Figure 3. MLPA-identified location of the deleted exon 45 of the fetal DMD gene.

CONCLUSION

We report a fetus with a single exon deletion of the DMD gene detected by routine CMA, which suggests a novel approach to preventing missed diagnosis and delivery of babies with exon deletion or duplication of the DMD gene.

Data Availability:
The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

Source of Funds:
Not applicable.

Declaration of Interest:
The authors declare that they have no conflict of interest.

References:


