

Unexpected DMD Gene Mutations Detected by CMA and CNV-seq in amniotic Fluid and Aborted Fetus Samples

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Research Article

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Abstract

Background: Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are common X chromosome-linked recessive hereditary diseases. The mechanism is that the exon mutations of anti-myotrophy protein gene (Dystrophin gene) and lead to muscle dysfunction. Prenatal diagnosis can prevent the birth of children with defects and have good clinical significance.

Methods: CMA and CNV-seq were used to detect the amniotic fluid after amniocentesis,. CNV-seq was used to detect spontaneous abortion tissue. The DMD gene mutations were found in 6 amniotic fluid samples and one spontaneous abortion sample. DMD gene mutations were confirmed by MLPA and new DMD mutations were found.

Results: CMA found DMD mutations :1.Xp21.1, 75.5kb del (E52-53); 2.Xp21.2, 334.92kb dup (E61-79); 3.Xp21.2, 292.25kb dup (E58-74); 4.Xp21.1, 374.20 kb dup (E45-51). CNV-seq found DMD mutations: 5.X p21.2, E64-79 dup; 6.X p21.1, E1-7dup; 7.Xp21.1, E 44-52 del.

Conclusions: 4 fetuses harboring DMD gene mutations were found by CMA, 2 fetuses and 1 induced abortion carrying DMD gene mutations was detected by CNV-seq. CMA/CNV-seq jointed with MLPA test can provide more comprehensive evidence for prenatal diagnosis.

Introduction

Dystrophin gene(DMD gene) is located in chromosome Xp21.1, mutations in this gene can cause DMD or BMD [1]. Most of the patients were male, DMD incidence among live-born boys is nearly 1/3500, the symptom of walking disability may emerge at 3~5 years old, getting worse and worse step by step when he grow up until losing ability to walk, died at about 30 years old because of heart and lung failure [2]. BMD incidence among live-born boys is about 1/20000, clinical manifestations are milder than DMD, generally have late onset age and longer life cycles. One of DMD/BMD problems is that patients with no family history can not usually be found before birth, but after the new technologies such as genome-wide high resolution microarray analysis (CMA) , single nucleotide polymorphism (SNP)and high-throughput next generation sequencing technologies (CNV-seq) are widely used in prenatal diagnosis [3-5], more and more minor structural abnormalities such as chromosome microdeletions and microduplications have been detected. There may even be some unexpected findings that do not totally match the patient's clinical indications,such as DMD/BMD. We found seven DMD gene mutations cases by CMA and CNV-seq accidentally, and then used MLPA (Multiplex ligation-dependent probe amplification) to confirm the mutations and found new mutations(Fig.1,Fig2).. The mutations may lead to fetal DMD or mild BMD after mutations analysis.

Materials And Methods

Samples

Patients with abnormal serological Down screening, B ultrasound abnormality or high NIPT(Non-invasive Prenatal Testing) risk came to our department for invasive prenatal diagnosis. The spontaneous abortion sample was send from the Department of Gynaecology and Obstetrics, Northwest Women's and Children's Hospital, Xi'an, China.(Tab.1). All methods of prenatal diagnosis were introduced to patients, consents were signed by patients, and the tests were selected by patients voluntarily.

aCGH test

Getting 10 ml amniotic fluid by amniocentesis, cells in amniotic fluid were collected by centrifugation and divided into two 1.5 ml EP tubes, one tube was used for experiments, the other was stored at -20°C. Using PureGene tissue extraction kit (Qiagen) to extract genomic DNA. Marking, purifying, hybridizing, washing and scanning the DNA according to Agilent 8×60 K chip detection process. Uploading the scanning data to Cytogenomics software (Agilent Technologies) and analyzing the scanning data preliminarily. At the same time, the scanning data was converted into .zip file and uploaded to Genoglyphix (Perkin Elmer), using public and laboratory databases such as DGV,DECIPHER,ISCA,OMIM,ClinVar,PubMed to analyze pathogenicity of CNVs fragment ≥100 kb. The CNVs were classified into three categories according to the 2015 ACMG guidelines :(1) Pathogenic CNVs; (2) Variant Unknown Significance (VUS) CNVs; (3) Benign CNVs.

CNV-seq

Getting amniotic fluid was described as in CMA test. The extraction of genomic DNA from amniotic fluid and skin of aborted fetus was taken by DNeasy Tissue Kit (69506) (Qiagen)[6]. Using Chromosome CNV Detection Kit KR0040 (Berry Genomics Corporation, Beijing, China) to detect CNV-seq. The procedure was performed as described before [7]: Nearly 50 ng DNA was hydrolyzed by restriction enzymes to obtain DNA fragments at an average size of 200 bp and established DNA libraries by small DNA fragments end filling, adapter ligation, and PCR amplification. To determine all sequences on the NextSeq 500 high-throughput sequencing platform (Illumina USA). Compared the sequences with h19 Human Genome Database by parallel alignment software and analyzed pathogenicity of CNVs fragment ≥ 100 kb as described in CMA test.

MLPA test

DMD Gene Detection Kit was SALSA MLPA kit DMD P034/P035(MRC company, Holland) ABI3500DX Genetic Analyzer(AB Applied Biosystems) was used for capillary electrophoresis, and Coffalyser NET software was used for data analysis.

Discussion

Mutations in DMD genes can lead to muscular dystrophy (DMD or BMD). If the deletion or duplication mutations in DMD gene destroys the reading frame and result in the anti-myotrophin protein content less than 3% or can't produce myotrophin, patients show DMD. If deletion or duplication mutations keep the reading frame intact or less destroyed, it leads to the anti-myotrophin protein keeping part of the function, patients show BMD. But there are also a few patients who DMD gene deletion or duplication did not alter the reading frame, it still show DMD. [8]

The main DMD gene mutation type of is partial deletion or repetition of 79 gene exons, nearly account for 60%~70% of all mutation types. The rest are point mutations, minor insertion or deletion mutations. Despite many studies on gene therapy in DMD, such as exon hopping, readout of nonsense stop codon or injection of vectors with full DMD gene, gene therapy still faces great challenges and dilemmas[9]. There is still no effective treatment for DMD/BMD patients until now [10,11], early diagnosis and early treatment can prolong the survival age and improve the quality of life of DMD patients, but can not be cured. DMD patients with no family history of hereditary diseases can not usually be found before birth [12-16]. A majority of DMD patients develop clinical symptoms around age 3. But the results of serum creatine kinase level, neuroelectrical trophysiological examination and DMD gene detection were clearly diagnosed until 5 years old, so genetic counseling and screening for DMD genes prior to or in early pregnancy are particularly important, prenatal diagnosis is an important approach to prevent the birth of the patient with a family history of DMD/BMD or with no history.

High-resolution genomic CMA and high-throughput next generation sequencing CNV were used in prenatal diagnosis not only detect chromosomal microdeletion and microduplication syndrome, but also helped to detect single gene disease caused by gene deletion or duplication, can improve the diagnosis rate of disease. In this study, the pregnancy of all male fetuses were terminated. Two female fetuses were born: Case6 is normal; but Case 3 showed upward bent legs and heavy hair, WES did not find problems. One female fetus (Case 5) was aborted. (data of 7 patients showed in Tab 1). We compared the DMD gene mutations between fetus and mother, found some mutations inherited from mother(Fig 1) and some didn't(Fig 2), it also reveals the importance of prenatal diagnosis because the mother is carrier or healthy woman, and with no family history of DMD. So it is beneficial to the correct evaluation of fetal prognosis in prenatal clinical consultation, and provide a more comprehensive advice to decide if the pregnancy should continue or be terminated to prevent the birth of children with defects.

Table 1

Clinical and experimental data of the 7 analyzed patients

Case	Weeks	Gender	Medical history	Sample	Results	MLPA	Inheritance	Follow-up
1	24+1	male	Omphalocele	amniotic fluid	Xp21.1(31691172_31766673)x0 (CMA)	E52-53 del	Y	abortion
2	30	male	1.right aortic arch 2.left artery vagal 3.left ventricular bright spot.	amniotic fluid	Xp21.2(31016983_31351900)x2 (CMA)	E61-79 dup	Y	abortion
3	32+1	female	1.NF:6.8mm 2.Down risk:1:413	amniotic fluid	Xp21.2(31182699_31474949)x3 (CMA)	E58-74 dup	Y	born with problems
4	31+3	male	sacroccocygeal vertebrae dysplasia	amniotic fluid	Xp21.1(31777925_32152126)x2 (CMA)	E45-51 dup	Y	abortion
5	24+5	female	Down risk:1:227	amniotic fluid	dup(X)(p21.2) chrX:g.30860000_31260000 (CNV-seq)	E64-79 dup	N	abortion
6	22+6	female	Sex Chromosome Number Variant (NIPT)	amniotic fluid	dup(X)(p21.1)chrX:g.32820000_33560000 (CNV-seq)	E1-7 dup	N	born
7	21+2	female	1.skin edema 2.scites 3.bilateral foot varus	aborted fetus	del(X)(p21.1) chrX:g.31760000_32000000 (CNV-seq)	E45-51 del	Y	

Declarations

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Author contributions

CL, QW conceived and wrote the manuscript.

Ethics approval and consent to participate

Approved by Ethics Committee of Northwest Women's and Children's Hospital

Consent for publication

All authors approved to publish. All patients signed the informed consent papers and approved to publish

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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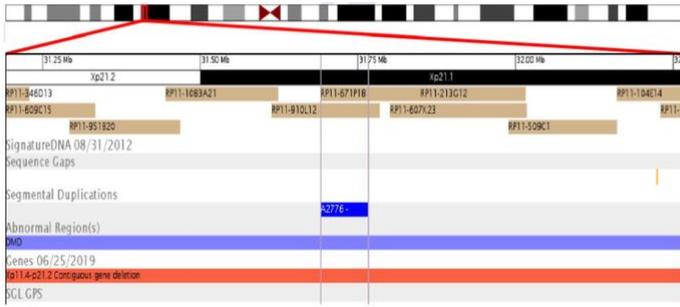
Not applicable

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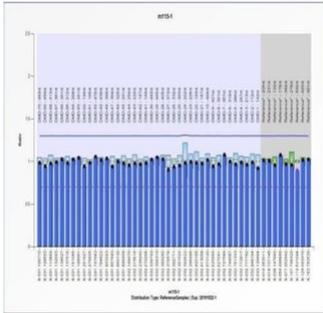
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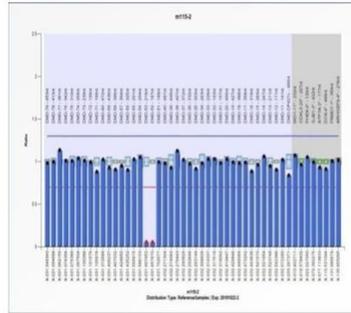
Figures



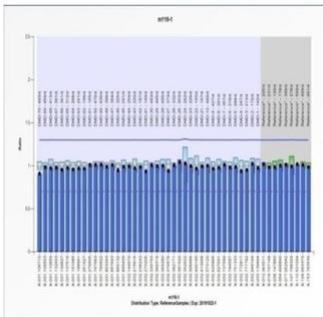
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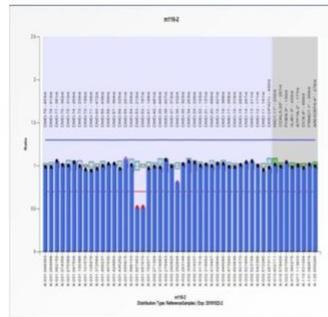
B



C



D



E

Figure 1

Results of case 1 A, Fetus CMA Result: Xp21.1(31691172_31766673)x0; B,C. Fetus MLPA result: E52-53del D,E. Mother MLPA result: E52-53del Mutations inherited from mother

