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**Early-onset ophthalmoplegia, cervical dyskinesia, and lower extremity weakness due to partial deletion of chromosome 16: A case report**



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**Abstract**

BACKGROUND

We explored the genotype-phenotype correlation of novel deletion of 16p13.2p12.3 in an 8-year-old child with progressive total ophthalmoplegia, cervical dyskinesia, and lower limbs weakness, by comparing the patient's clinical features with previously reported data on adjacent copy number variation (CNV) regions.

CASE SUMMARY

Specifically, we first performed whole-exome sequencing (WES), CNV-sequencing (CNV-seq), and mitochondrial genome sequencing on the patient and his parents, then applied "MitoExome" (the entire mitochondrial genome and exons of nuclear genes encoding the mitochondrial proteome) analysis to screen for genetic mitochondrial diseases. We identified a *de novo* 7.23 Mb deletion, covering 16p13.2p12.3, by both WES and CNV-seq. We also detected 16p13.11 in the deleted region, which is the recurrent distinct region associated with neurodevelopmental disorder (NDD). However, the patient only displayed features of progressive total ophthalmoplegia, cervical dyskinesia and weakness in his lower limbs without NDD. The "MitoExome" sequencing was negative. Brain magnetic resonance imaging revealed non-specific sporadic changes in occipital parietal lobe and basal ganglia.

CONCLUSION

Taken together, these results indicated that 16p13.2p12.3 deletion causes a syndrome with the phenotype of early-onset total ophthalmoplegia. The "MitoExome" analysis is powerful for the differential diagnosis of mitochondrial diseases. We report a novel copy number variant in this case, but further confirmation is required.

**Key Words:** Cervical dyskinesia; Copy number variation; Lower limbs weakness; Ophthalmoplegia; Whole-exome sequencing; Case report

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**Core Tip:** At present, little is known about the associated phenotypes of copy number variations (CNV) in the short arm of chromosome 16. The most remarkable CNV is 16p13.11 microdeletion/microduplication. The main clinical features of this syndrome are a series of neurological abnormalities such as mental retardation, autism, schizophrenia, epilepsy and attention deficit hyperactivity disorder. We identified a de novo 7.23 Mb deletion, covering 16p13.2p12.3. 16p13.11 was included in the deleted region, which is the recurrent distinct region associated with neurodevelopmental disorder (NDD). However, the patient only displayed features of progressive total ophthalmoplegia, cervical dyskinesia and weakness in lower limbs without NDD.

## INTRODUCTION

At present, little is known regarding the associated phenotypes of copy number variations (CNV) in the short arm of chromosome 16. The most remarkable CNV is 16p13.11 microdeletion/microduplication. Notably, nudE nuclear distribution gene E homolog 1 and N-terminal asparagine amidase genes located in 16p13.11 region have been associated with a series of neurological abnormalities, such as intellectual disabilities, autism, schizophrenia, epilepsy and attention-deficit hyperactivity disorder

in patients with this syndrome[1,2]. The adjacent regions of 16p13.11, chromosome 16p13.3 deletion causes a syndrome, which is characterized as failure to thrive, hypotonia, short stature, microcephaly, characteristic facial features, mild to moderate intellectual disability, organ anomalies, and vulnerability to infections[3]. Previous studies have shown that chromosome 16p11.2 Locus, the other neighboring region characterized by recurrent CNVs, is the chromosomal region related to Autistic Spectrum Disorder[4,5]. It seems that 16p13 and its adjacent regions are the highly suspected sites associated with a series of neuropsychiatric disorders. Primary or secondary causes of internal ophthalmoplegia usually indicate a central nervous system situation. For example, patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome, may display primary encephalopathy, including ptosis (external ophthalmoplegia) with internal ophthalmoplegia[6]. Notably, MELAS syndrome is caused by mitochondrial DNA (mtDNA) mutations, while mitochondrial variations have been attributed to occurrence of diseases of Kearns-Sayre syndrome (KSS), which is associated with early-onset ophthalmoplegia[7,8].

## CASE PRESENTATION

### *Chief complaints*

The patient was an 8-year-old boy, who presented with gradually progressive unilateral ptosis at the age of 6 years, which developed to bilateral ptosis at 8 years and 4 mo.

### *History of present illness*

The patient was an 8-year-old boy with bilateral ptosis.

### *History of past illness*

At the end of December 2017, the patient developed a fever of unknown origin, with his body temperature found to be as high as 38.5 °C. He recovered after being given oral antipyretics. One week after the fever, the patient had a stroke of dyskinesia and kept

tilting his head to the left (cervical dyskinesia), a phenomenon that was accompanied by eye movement disorders.

***Personal and family history***

The patient was the first child born to non-consanguineous parents, and there are no similarly affected family members. Although he had a "history of congenital heart disease", subsequent cardiac color Doppler ultrasound examination revealed normal results. The patient had no developmental milestone delays.

***Physical examination***

Physical examination revealed that he had bilateral upper eyelids dropping (50% pupils covered). Moreover, he had limited abduction in the right eye, neck dystonia, slightly reduced (degree 5-) muscle strength in bilateral lower limb, while his bilateral knee reflexes disappeared. He also exhibited right cryptorchidism, and a small penis. He could neither squat nor jump on one foot.

***Laboratory examinations***

Blood tests showed unremarkable results. Notably, he had a blood acetylcholine receptor (anti-AChR) antibody level of 0.82 nmol/L, while IgG tests for anti-MuSK, anti-Titin antibody, and human low-density lipoprotein receptor-related protein 4 were negative.

***Imaging examinations***

Brain magnetic resonance imaging (MRI) revealed diffused signals in the occipital, parietal cortex and basal ganglia (Figure 1). His right ptosis was better, although it did not disappeared after treatment with prednisone, neostigmine, and omeprazole from January 2018 to October 2019. His eye lid dropping re-appeared in March 2020 and progressed to be bilateral. in August 2019, he was hospitalized due to a recurrent headache, and was unresponsive to neither mannitol nor carbamazepine. The patient is currently in the first grade of elementary school with medium grades.

## WHOLE-EXOME SEQUENCING

Whole exome libraries were prepared using the xGen Exome Research Panel v1.0 (IDT, Iowa, USA), and sequenced on the Novaseq 6000 platform (Illumina, San Diego, CA, USA). Raw data were cleaned using the fastp software package. Subsequently, the paired-end reads were performed using Burrows-Wheeler Aligner (BWA) to the Ensemble GRCh37/hg19 reference genome. synonymous and short indel calling were conducted using GATK software package, followed by ANNOVAR annotation. Prediction was performed using the Provean, Sift, Polypen2_hdiv, Polypen2_hvar, Mutationtaster, M-Cap, and Revel software packages. The pathogenicity of all the variants was interpretated according to the guidelines of the American College of Medical Genetics and Genomics (ACMG).

## COPY NUMBER VARIATION SEQUENCING

We performed CNV-seq[9], a CNV detection method based on high-throughput sequencing, in the patient. Briefly, genomic DNA was first sheared to 200-300 bp fragments *via* sonication, then subjected to quality control *via* electrophoresis. Ends of DNA fragments were patched using DNA repair enzyme system to generate blunt ends, then a single adenine (A) nucleotide added to the 3' end to form an overhanging A-tail. Subsequently, the genome was amplified by Ligation-mediated polymerase chain reaction (LM-PCR) for 4-6 cycles. We used the same sequencing platform and data cleaning protocols to detect CNVs with a length of 100 KB and above using Chigene independently developed software packages. After that, we employed Decipher, ClinVar, OMIM, DGV, and ClinGen for annotation.

## HIGH-SENSITIVE MITOCHONDRIAL DNA SEQUENCING

At least 2 mL peripheral blood were collected from the patient, and mtDNA extracted using the mitochondrial DNA extraction kit. Full-length mitochondrial DNA was amplified and purified *via* PCR, using the high-fidelity DNA polymerase and visualized

*via* agarose gel electrophoresis. Paired-ended 150 bp (PE150) sequencing was performed on the Novaseq6000 sequencing system. The sequenced data was aligned to reference sequence of NC_012920 (human complete mitochondrial genome 16569 bp circular DNA) using the BWA software. The variants were then mapped onto the MITOMAP database while pathogenicity was performed according to the MITOtip.

## GENE TESTING RESULTS

WHOLE-EXOME SEQUENCING (WES) sequencing results revealed no suspected disease-cause variants. Next, we employed the CNV analysis method (developed by Chigene) on WES data and found that 2 deletions of neighboring region of Chr16:15,125,591-16,326,688(~1.20 Mb) and 9857005-14989502(~5.13 Mb). CNV sequencing data revealed *de novo* heterozygous deletion of chr16:9,699,585- 16928372 (~7.23 Mb) (Figure 2). Next, we compared this region and central nervous system phenotype with Decipher patients previously documented (Figure 3). Genes related to OMIM disorders are listed in Table 1. The mtDNA sequencing revealed negative results.

## FINAL DIAGNOSIS

A *de novo* heterozygous deletion of chr16:9699585-16928372 (~7.23 Mb) was identified. Due to this deletion on chromosome 16, the patient has early-onset ophthalmoplegia, cervical dyskinesia, and lower extremity weakness.

## TREATMENT

Since the patient showed elevated anti-AChR antibody levels, treatment with glucocorticoids and an acetylcholinesterase inhibitor was used.

## OUTCOME AND FOLLOW-UP

The treatment effect was good.

## DISCUSSION

Ophthalmoplegia cases are characterized by central neurological, muscular, and synaptic abnormalities. In the present case, the evidence of the stroke-like onset of internal ophthalmoplegia and cervical dyskinesia, and brain MRI findings revealed a central nervous system disorder. Ophthalmoplegia, usually caused by damage to the midbrain. This patient's MRI revealed no abnormal signals in his midbrain and middle cranial fossa. In the patient's anamnesis, we originally considered ophthalmoplegic migraine (OM) or recurrent ophthalmoplegic neuropathy (RPON), a rare condition manifesting as episodes of ipsilateral headache followed by ocular cranial nerves palsy, owing to his had unexplained headache during the treatment. However, previous studies have shown that ophthalmoplegia typically persists for weeks to months and was reversible in patients with OM/RPON[10,11]. Since the patients displayed elevated anti-AChR antibody levels and responded well to treatment using glucocorticoid and acetylcholinesterase inhibitor, myasthenia gravis (MG) was also the candidate diagnosis. However, MG failed to explain the early and urgent pattern of onset, as well as the brain changes.

Mitochondrial diseases characterized by ocular symptoms include chronic progressive external ophthalmoplegia (CPEO) and KSS. CPEO is a relatively mild mitochondrial disease characterized by extraocular muscle weakness and ptosis, often with weakness in the extremities[12]. Ptosis usually progresses to ophthalmoplegia over months or years. KSS was defined as CPEO initiated before age 20 with retinopathy of pigmentation and associated with at least one of the following: cardiac conduction disturbances, cerebellar ataxia, or elevated cerebrospinal fluid protein concentrations[13]. KSS, mitochondrial cytopathy due to mitochondrial CNV[14], could explain the early-onset, progressive external ophthalmoplegia and suspected cardiomyopathy. However, analysis revealed negative results.

Our patient exhibited different clinical features from the previously reported cases of chromosome 16p (chr16p) CNVs. As far as we know, CNVs in chr16p, including the proximal 16p11.2 deletion/duplication, intermedia 16p13.11 microdeletion/microduplication and terminal 16p13.3 deletion, have been strongly associated with neurodevelopmental and/or neuropsychiatric disorders[1-5]. Moreover,

Redaelli[15] identified four smallest regions of overlapping (SROs), one located in 16p13.11, one located in at 16p12.2, and two close SROs occurring in 16p11.2. Based on the results of 27 CNVs in chr16, the authors concluded that developmental delay (DD) in those patients was caused by recurrent non-allelic homologous recombination in chr16[15]. Notably, the CNV in our patient resulted from full-length deletion of 16p13.11, while it is supposed to be associated with signs of 16p13.11 microdeletion syndrome, that is developmental delay, microcephaly, epilepsy, short stature, facial dysmorphism and behavioral problems. However, we found none of these signs in our patient. Summarily, identification of the novel pathogenic CNV in our patient was inconclusive, according to the documented cases and OMIM genes (Table 1).

## CONCLUSION

In conclusion, we identified a CNV heterozygous deletion in the 16p region in a Chinese patient with premature ophthalmoplegia, cervical spine dyskinesia, and lower extremity weakness through whole-exome sequencing, CNV seq and mitochondrial exome (MitoExome) analysis strategies. MitoExome analysis is critical for differential diagnosis of mitochondrial diseases. Particularly, ptosis, ophthalmoplegia, and proximal muscle weakness are common occurrences in both mitochondrial diseases (MDs) and MG, which strongly indicate neuromuscular junction (NMJ) disorders. Generally, clinical grounds alone cannot effectively distinguish MDs and MG in patients with NMJ dysfunction[16,17]. Since NMJ abnormalities are common in MDs, there is need to perform MDs-associated genetic tests. We recommend that “MitoExome” analysis should be applied in patients with ambiguous diagnosis of NMJ disorders.

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