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Chromosome 22q11.21 and 11p15.4 microdeletions confirmed by DNA high throughput sequencing analysis in one patient with asymmetric cry syndrome:Case report and review of the literature

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Abstract

The case report describes a newborn case of ACS based on clinical presentation(s) and microdeletions of 22q11.21 and 11p15.4 using DNA high throughput sequencing analysis. This finding has implications for ACS diagnosis and overcomes the limitations associated with FISH by using the DNA high throughput sequencing across the whole genome.

Key Clinical Message

Apart from a microdeletion of chromosome 22q11.21, a novel microdeletion of chromosome 11p15.4 was found in a newborn case of ACS using the DNA high throughput sequencing across the whole genome.

1. Introduction

Asymmetric cry syndrome (ACS) is a genetic abnormality and is one of the recognized disorder of chromosome 22q11.2 deletion syndrome (22q11.2 DS). It was first described by Cayler (Cayler, 1969) in 1969, hence the name "Cayler cardiofacial syndrome". A microdeletion of chromosome 22q11.2 is found in most of the patients with conotruncal anomaly face syndrome, Di George's syndrome and velocardiofacial syndrome. Patients with 22q11.2 deletion syndrome may have several clinical abnormalities and different degrees of organ commitment, which include thymus dysfunction, cardiac diseases, immunodeficiencies, and other clinical problems (Kobrynski & Sullivan, 2007). ACS is a rare syndrome with asymmetric crying faces (ACF) in patients with congenital heart diseases.

The age at presentation of ACS varies according to specific anomalies in the patient. ACS in neonates is a rare condition and 22q11.2 deletions often manifest as clinically significant conotruncal defects, such as subaortic stenosis with malalignment of infundibular septum, truncus arteriosus, and tetralogy of Fallot (Hanneman, Newman, & Chan, 2017).

ACS is a rare disease in China. Previously, no heart murmurs were reported to be associated with the condition, and the facial phenotypical features were so trivial such that the patients were thought to be normal by their parents until a diagnosis was made. Thus, early diagnosis by fluorescence in situ hybridization (FISH) might be more difficult when a case has no evidence of chromosomal deletion of 22q11.2. According to a recent study, 31% of patients with 22q11 deletion syndrome (DS) were not diagnosed till when they are 10 years of age (Friedman, Rienstein, Yeshayahu, Gothelf, & Somech , 2016).

To make an early diagnosis, it is important for clinicians to be aware of this syndrome and to familiarize themselves with related extracardiac manifestations. 22q11.2 DS is traditionally diagnosed with FISH. Array

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comparative genomic hybridization (aCGH) is considered as one of the alternatives in diagnostic modalities (Bahamat et al., 2018). Application of high-throughput pyrosequencing to comprehensively analyze microdeletions of 22q11.2 DS has not yet been reported in the literature. Earlier studies reported that Cayler Cardio-facial syndrome involves 22q11.2 DS, in which a small part of chromosome 22 was missing. Hence, in this case report, a novel ACS with 22q11.21 and 11p15.4 microdeletions, confirmed by DNA high throughput sequencing analysis is presented.

2. Clinical Case Presentation

A 10-minute-old newborn male baby was admitted to our neonatal intensive care unit (NICU) department due to dyspnea. The baby was born to a 20 years old G1P1 preeclamptic (treated) mother at 34 weeks and 6 days of gestation through cesarean section and weighed 1.8 kg (weight centile less than the 10th). The baby had an Apgar score of 7-8 at 1 minute and then 5 minutes and was transferred to the NICU due to increasingly worsening condition. There was no maternal infection(s) and no family history of hereditary, neurological, or systemic diseases were reported from the baby's paternal or maternal side.

On examination, the baby was fully conscious with a body temperature of 35.8°C, heart rate of 130/min and respiratory rate of 58/min. Breathing sounds were clear and heart beat was regular, without any murmurs. There was nothing abnormal detected on neurological examination. Further inspection revealed no facial abnoamlities, no cleft lip or palate, no low set or overfolded ears, no hypertelorism, no narrow palpebral fissures, no limb anomalies like syndactyly and polydactyly, no hypospadias, and no imperforate anus.

Due to the increasingly worsening condition, the infant was treated with nasal continuous positive airway pressure (NCPAP). In addition, natural bovine surfactant with 100 mg/kg of phospholipids was given through "endotracheal tube" to treat the respiratory distress syndrome (RDS) as manifested on the chest X-ray. Follow-up examination showed improvements in the saturations

The baby continued to perform well on gentle invasive ventilation, maintaining saturations of above 90%. On day 7 of admission, a heart murmur was discovered during routine physical examination. Meanwhile, his lower lip was pulled downwards deviating towards the left during a crying episode, but there was no facial asymmetry during sleeping or at rest (Figure 1). Furthermore, the baby had no difficulty in closing eyes and had normal forehead wrinkling and sucking movements which ruled out a possibility of facial nerve palsy. With these symptoms, ACS was diagnosed and so further investigations were performed. Cranial ultrasound showed a grade 1 periventricular hemorrhagic infarction (PVHI) and bilateral ventriculomegaly. Additionally, echocardiogram revealed membranous ventricular septal defect (5.5mm), multiple atrial septal defects (2.0mm and 1.4mm), and mild pulmonary arterial branch stenosis. The baby was examined with DNA high throughput sequencing analysis, which showed microdeletions of chromosomes 22q11.21 and 11p15.4 (Figure 2). Notably, a 2.92 Mb of chromosome 22q11.21 (18880001-21800000) was deleted, which included 75 RefSeq genes. In addition, a 0.12 Mb of chromosome 11p15.4 (10080001-10200000) was deleted, which contained 1 RefSeq gene.

After using pulmonary surfactant for one week, the baby showed quick and progressive clinical improvement. From days 8 to 12, he underwent full enteral feeding by increasing volumes of milk. He was then discharged in a healthy condition and has been on regular follow up since that time.

3. Discussion

The general features of 22q11.2 DS vary widely (more than 180 phenotypic presentations) and includes Di George's syndrome, Shprintzen's syndrome as well as Cayler cardio-facial syndrome. The syndromes were named separately after their first description by the author. Later, with the advancements in the diagnostic methods in the field of genetics, it was found that 22q11.2 chromosome was deleted in all these syndromes. They are currently grouped under "the 22q11.2 deletion syndromes", as it is difficult to choose a single term.

22q11.2 DS is one of the most frequently encountered interstitial deletion syndromes in the population, with an estimated frequency of 1/4000-5000 (Scambler, 2000). Though usually sporadic and autosomal dominant inheritance, it has been reported in 10-20% of the patients (Bassett et al., 2011).

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The chromosome 22q11 region is very unstable with misalignment of chromosome-specific low-copy repeats (LCR22A-H) during nonallelic homologous recombination, leading to the deletion of 22q11.2 region (Burnside, 2015). Approximately 90% of patients with 22q11DS have a 1.5˜3Mb deletion (Burnside, 2015; Yagi et al., 2003). The haploinsufficiency of genes located at 22q11.2, especially *TBX1* , can disturb the early morphogenesis of many organs including the thymus, parathyroid gland and facial structures. This explains as to why there are wide clinical manifestations like cardiac defects, thymic hypoplasia, abnormal facial features, cleft palate, and hypocalcemia associated with it (Yagi et al., 2003).

The cause of hypoplasia of the depressor anguli oris muscle still remains to be elusive, though previous studies postulated intrauterine molding or subclinical viral infections as the cause. This finding needs further confirmation. Lahat et al. (Lahat, Heyman, Barkay, & Goldberg, 2000) reported a prospective study, wherein 17 out of 5532 infants had ACF. There were no noxious obstetric perinatal factors identified and no family history of hereditary diseases in all the cases. In our case, we also found no maternal infection and no family history of hereditary or systemic diseases associated with ACS.

Hypoparathyroidism and hypothyroidism are commonly observed in patients with 22q11.2 deletion and requires much attention. Hypoparathyroidism is the first hormonal disturbance recognized in DGS and is documented by aplasia and hypoplasia of the parathyroid glands during surgery or autopsy. Furthermore, hypocalcemia can occur transiently during the neonatal stage with symptoms of seizures, tremors, or tetany, which mainly occur due to low parathyroid reserve and abrupt cessation of maternal calcium supply after birth. In our case, serum calcium level was normal (2.3mmol/L, reference rage, 2.1-2.7 mmol/L), no hypocalcemic symptoms of seizures, tremors, or tetany were observed on admission, which ruled out the possibility of hypocalcemia due to hypoparathyroidism. Although hypocalcemia may not be present during the neonatal period (Pawar, Sharma, Srilakshmi, Reddy Chejeti, & Pandita, 2015), studies have shown that it can occur at any time during childhood (Friedman, Rienstein, Yeshayahu, Gothelf, & Somech. 2016; Fu, Leung, Kao, & Yeh, 2015), adolescence (Yoo, Kim, Cho, Kwon, & Yoo, 2017) and even in adulthood (Fung et al., 2015; Maldjian & Sanders, 2018). This is likely due to the recurrence of hypoparathyroidism precipitated by increased metabolic demand and acute illness during pregnancy, surgery, infection, or any physiologic stress conditions. Regular lifelong follow-up of calcium, magnesium, and PTH levels are required in patients with 22q11DS (Cheung, George, Costain, Andrade, Chow, Silversides, & Bassett, 2014). Calcium and vitamin D supplements are recommended to patients with 22q11DS, regardless of whether they have been diagnosed with hypocalcaemia or not. However, iatrogenic hypercalcemia resulted in renal calculi and renal failure, and so should be avoided (Fung et al., 2015).

Immunodeficiency has been reported in 80% of the patients with 22q11 syndrome. As a result of thymic hypoplasia, cell-mediated immunity is usually involved with 22q11DS, decreasing the T-cell numbers and functions. However, disorders of humoral immunity might also occur. Waters et al. (Waters, Peterson, & LaRussa, 2007) reported a case of pneumonia in a 13-month-old male child with partial DGS, and the child died after inadvertently receiving live viral vaccines. Recently, Matsuoka (Matsuoka et al., 2019) reported the first case of a teenage patient with chromosome 22q11.2 DS who died due to overwhelming post-splenectomy infection (OPSI) by *Streptococcus pneumoniae*despite appropriate prevention by pneumococcal vaccine. As such, it is appropriate to check immunoglobulin levels in the patient before vaccinations.

Congenital heart defects are one of the main clinical features of 22q11DS. Although serious cardiac anomalies were present in most of the patients in earlier studies, their prevalence accounted for about 40% according to the recent papers (Fung et al., 2015). Interestingly, our patient had ventricular septal defect and two atrial septal defects which warranted ongoing follow-up visists.

Some follow-up studies revealed that the frequency of psychomotor retardation and speech disorders was increased (Cancrini et al., 2014). However, information regarding long-term outcomes and older age ranged in ACS has been limited in China. Our patient showed a normal neonatal behavioral neurological assessment (NBNA) score at a corrected gestational age of 41 weeks and his physical index was between P10-P90, but he still had ACF. During the final evaluation, he was aged 2 years 6 mo, had developmental delay (height: 80cm; and body weight: 11kg) and speech deficits. However, the patient's intellectual level, cognitive and

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adaptive functioning and motor function were normal. The patient had undergone intracardiac repair the prior month which necessitated ongoing follow-up visits. The best case scenario involves early interventions for developmental delay and learning difficulties. Parents required counseling due to long-term outcomes, as deletion of 22q11.2 is associated with learning difficulties and mental retardation.

Previously, karyotyping results by GTG banding were normal in most of the ACF patients. Recently, more sophisticated techniques such as FISH have been replaced by karyotyping studies in most of the laboratories. It is therefore not surprising that several ACF cases with chromosome 22q11 microdeletions have been or continue to be reported (Pawar, Sharma, Srilakshmi, Reddy Chejeti, & Pandita, 2015). Some authors, therefore, suggested that newborns with ACF require additional screening of 22q11.2 DS (Pasick, McDonald-McGinn, Simbolon, Low, Zackai, & Jackson, 2013). In our case, genetic investigations were performed to exclude any underlying syndrome caused by 22q11 deletion. Notably, DNA high throughput sequencing analysis revealed two microdeletions of chromosomes 22q11.21 and 11p15.4, respectively. This, coupled with the clinical presentation confirmed the diagnosis of 'ACF' as a result of hypoplasia of the depressor anguli oris muscle.

Previous studies have reported that children diagnosed with Cayler Cardio-facial syndrome have an underlying condition called 22q11.2 DS, in which a small part of chromosome 22 is absent. More importantly, we detected additional chromosomal microdeletions in 11p15.4 in our patient. ACS is traditionally diagnosed using FISH with commercial probes. It is extremely accurate but limited to only one single-target sequence. Interestingly, our results showed the effectiveness of DNA high throughput sequencing, which overcame the limitations of FISH in terms of diagnostic yield and allowed whole genome screening and detection of a larger number of deletions and/or duplications in ACS patients.

In conclusion,our report reinforces that facial phenotypic and cardiac anomalies are manifestations associated with ACS. However, confirmation of this disease requires further genetic investigations. Apart from a microdeletion of chromosome 22q11.21, a novel microdeletion of chromosome 11p15.4 was found in our case. We, therefore, suggest that newborns with ACS should be screened with DNA high throughput sequencing analysis across the whole genome, which is more advantageous over FISH technique and could contribute to further research.

Authors' Contribution

Yonghong Pang was responsible for CNV sequencing data analysis and interpretation of sequence variants and drafting the manuscript; Yang Yu managed the patient; Xiaoyi Deng designed the report; Qian Liu and Junmei Yan were involved in the acquisition, analysis and interpretation of clinical data; Xiangyu Gao was responsible for manuscript editing. All authors have reviewed the manuscript and approved the final version to be submitted.

Conflict of Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

We obtained the informed consent for information of the parents in this study.

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Figure Legends