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A clinical characteristics and genetic analysis of a case of Rubinstein-Taybi syndrome with glaucoma

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Abstract. – The aim of this study was to analyze the clinical features of a Rubinstein-Taybi syndrome (RSTS) case with neonatal glaucoma. We also wanted to explore the manifestation of the disease in combination with genotype-phenotype correlation. For DNA extraction we used 2 ml peripheral blood, collected from the child and parents. The extracted genomic DNA was used for clinical exome sequencing. A 38-day old baby boy was diagnosed with congenital glaucoma on the third day after birth with symptoms, including choking milk, feeding difficulties and slow weight gain. He was admitted to the neonatology department because of lung infection. The clinical exome sequencing showed that the child has a c.2368C>T heterozygous mutation in exome 13 in CREBBP (cAMP responsive element binding protein) while his parents have no such mutation. Combining genetic data with the clinical features, this infant was diagnosed with RSTS. This is the first report of RSTS caused by a c. 2368C>T mutation in CREBBP. RSTS is an extremely rare disease with extensive clinical manifestations. It is highly overlapped with other syndromes which makes the diagnosis difficult. RSTS is easy to be missed or misdiagnosed due to the lack of specific clinical manifestations during the neonatal period. Neonatal specialists need to enhance their awareness and recognition of this condition, and use genetic testing as an effective tool in order to finalize their diagnosis.

Key Words:
Rubinstein-Taybi syndrome, CREBBP gene, Glaucoma, Newborn.

Introduction

Rubinstein-Taybi syndrome (RSTS) aka Broad thumb-hallux syndrome is an extremely rare syndrome with multiple congenital malformations and mental retardation. It has an estimated prevalence of one case per 100,000-125,000 live births[1,2]. The typical clinical features include growth retardation after birth, microcephaly, specific facial features, broad thumb and big toes, and mental retardation. At present, there is no clear diagnostic standard. In this study, a case of congenital glaucoma was diagnosed on the third day after birth. We believe that it was the youngest reported case of glaucoma in the current literature. Subsequently, this patient was further diagnosed with a new mutation on CREBBP gene, which causes RSTS. This study explored the relationship between the clinical characteristics and gene mutations of the child.

Case Report

The patient, a 38 day-old male (G1P1, 39 weeks gestation), was admitted to the hospital due to "found glaucoma for 35 days, choking and difficulties in feeding for 10 days". His birth weight was 2.65 KG and the length was 49 cm. He was not suffocated at birth and was breastfed after birth. The baby could be easily choked by milk, however, his feces and urine were normal. On the third day after birth, the family discovered that the infant had corneal turbidity and edema. After the admission to the Ophthalmology Department, a significant increase in intraocular pressure was discovered, and glaucoma was considered as the root cause of this condition. Brinzolamide eye drops was prescribed in order to reduce intraocular pressure. The intraocular pressure was re-evaluated three times a week and was gradually controlled. The child suffered from choking along with difficulties in feeding for 10 days before admission, and the family did not make any special treatment. On the day of admission in the ophthalmology hospital, chest radiography was

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performed, and fundus angiography surgery was scheduled. Chest radiography revealed the presence of a lung infection which caused his transfer to neonatology department in our hospital. The overall condition of the patient was suboptimal since the onset. The parents were in good health. They were not intermarriage and had no historical genetic diseases in their families. There were no abnormalities during maternal pregnancy and no perinatal complications.

Physical examination on admission: T: 37.0°C, P: 121 beats/min, R: 48 beats/min, BP: 74/42 mmHg, WT: 3.2 kg, head circumference 35 cm, poor developmental, hoarse cry, moaning, sardonic feature, tiny jaw, pale face. The breath sounds of both lungs were thick, and wet rales can be heard on both sides. The heart sound was powerful, with the III/VI rumble-like murmur. The abdomen was soft, the liver and spleen were normal, and the bowel sounds were normal. The thumb was thick, the right hand had a coherent palm, and the first toe was broad (Figure 1). The extremities can move freely. The limbs had good muscle strength and low muscle tension. The left testis had not declined, and the right testis was normal.

Blood-RT, urine-RT, stool-RT, immune globulin and thyroid function tests were normal. Blood biochemical showed ALT115.6 u/l; AST 201.4 u/l was normal. TORCH: CMV-IgM was positive. Blood, alveolar lavage fluid, urine CM-

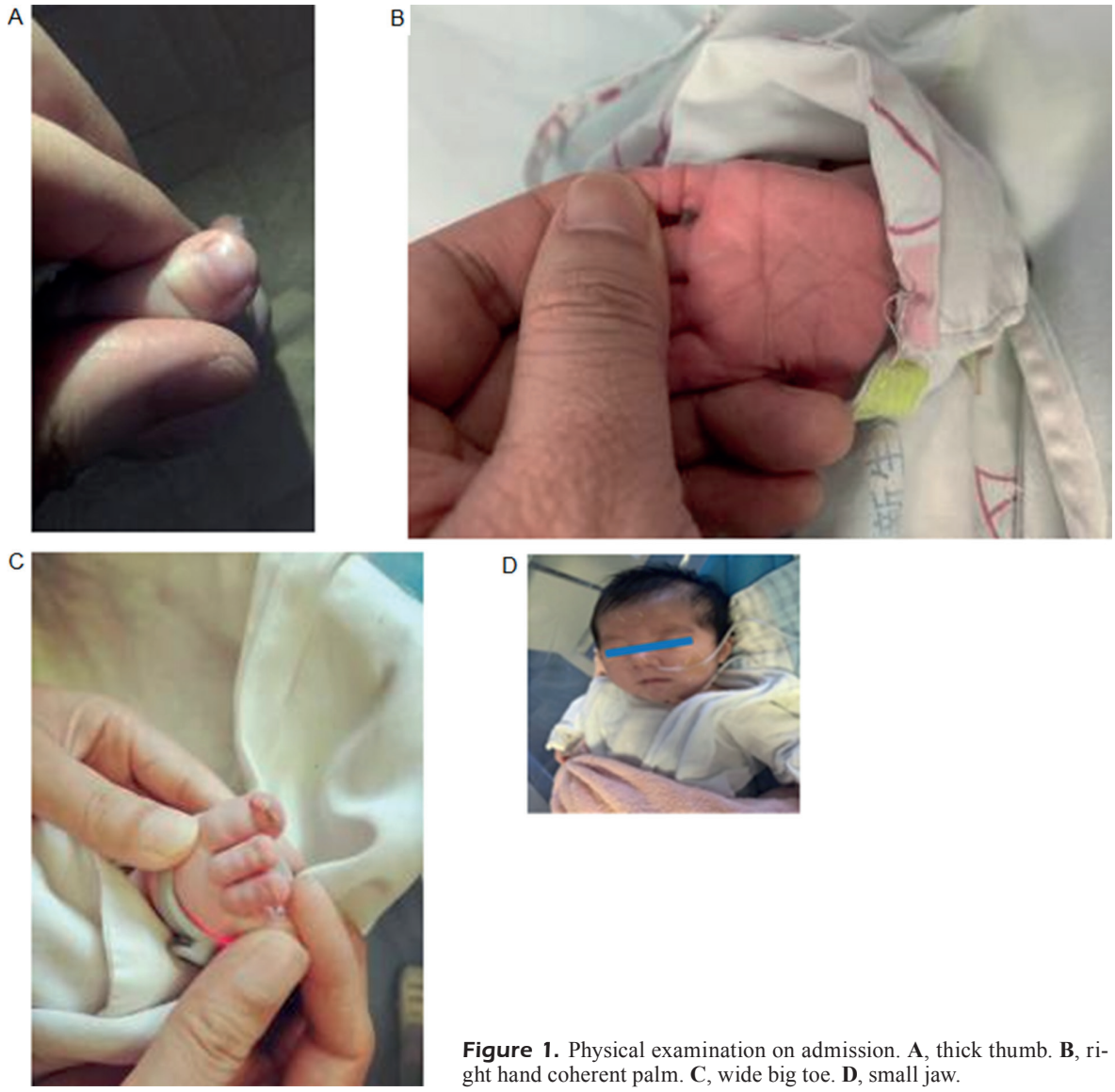

Figure 1. Physical examination on admission. **A**, thick thumb. **B**, right hand coherent palm. **C**, wide big toe. **D**, small jaw.

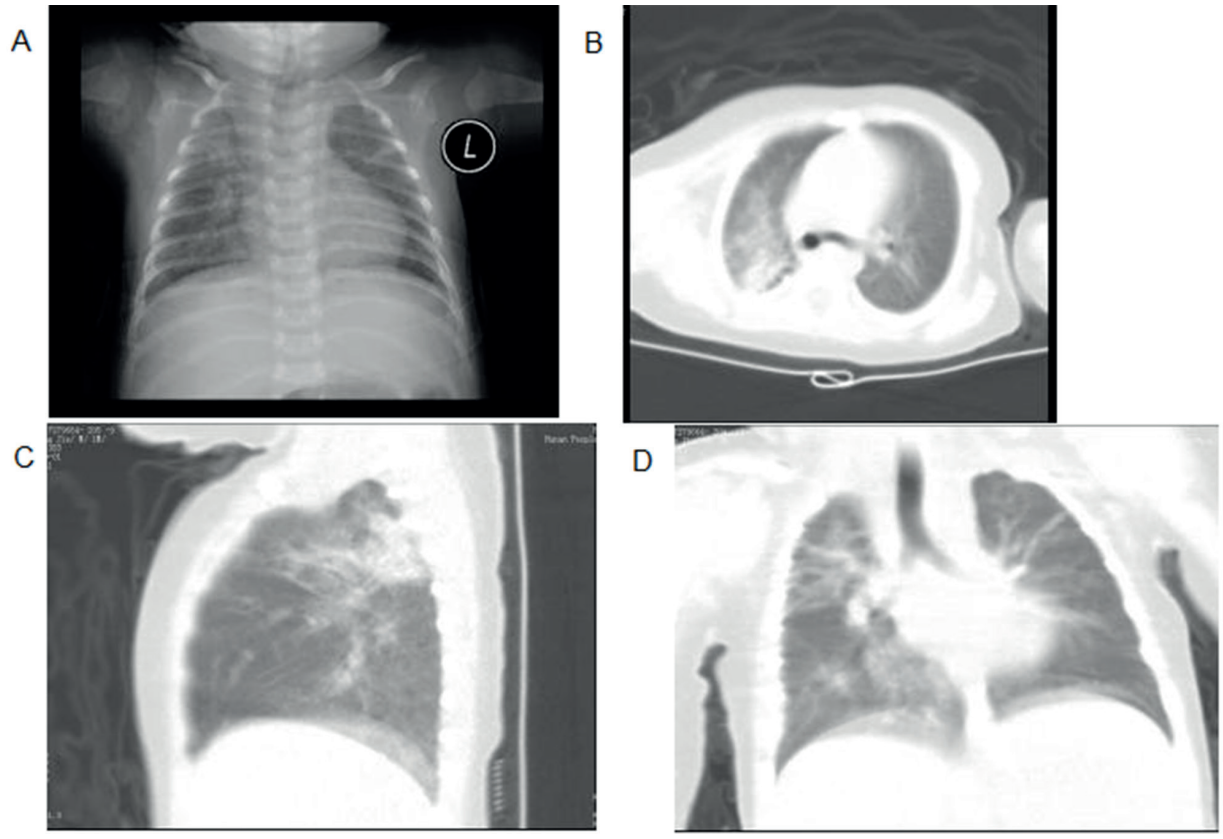

Figure 2. The two lungs are more divergent in the patchy density increase**. A-D**, Chest radiographs and lung CT were mostly scattered in the patchy density increase.

V-DNA were positive. CRP was 39.5 mg/l, PCT was normal, no abnormalities in blood culture, sputum culture, lung lavage fluid bacterial culture, cerebrospinal fluid culture. There was no abnormalities in cerebrospinal fluid routine. Chest radiographs and lung CT were mostly scattered in the patchy density increase (Figure 2); fiberoptic bronchoscopy showed tracheal softening, bronchial endometritis, and soft palate epiglottis softening (Figure 3). The electrocardiogram showed sinus tachycardia. Echocardiography revealed that the oval foramen was not closed, and the arterial catheter was not blocked. The results of B-ultrasound examination of the liver, spleen and urinary system was normal, while no abnormality on the head was detected with MRI. Ambulatory electroencephalogram was basically normal; hearing screening and brainstem evoked potential were found normal; hematuria genetic metabolism was normal.

Genetic testing was performed with the informed consent of the parents. We collected 2 mL of peripheral venous blood from the child and his parents in EDTA anticoagulation tubes. Samples were sent to a third-party testing facility for the Agilent V6 chip for full external capture. Illumina HiSeq2500 high-throughput sequencing was used. After the sequencing, data were evaluated and captured by Illumina Sequence Control Software (SCS). The results showed that the exon 13 of CREBBP gene in the child had a c.2368C>T heterozygous mutation (Figure 4). This mutation is a nonsense mutation in which a stop codon replaces a glutamic acid at 790th codon of CREBBP gene (p.Q790*), although neither of the parents had this mutation. The mutation was not reported in the HGMD and Clinvar databases. It was determined as a pathogenic mutation by ACMG guidelines. The genetic test results were consistent with clinical symptoms and patient was diagnosed with Rubinstein-Taybi syndrome.

Discussion

At the beginning, RSTS was diagnosed through clinical symptoms and imaging results. In 1991, for the first time, new mutations in the

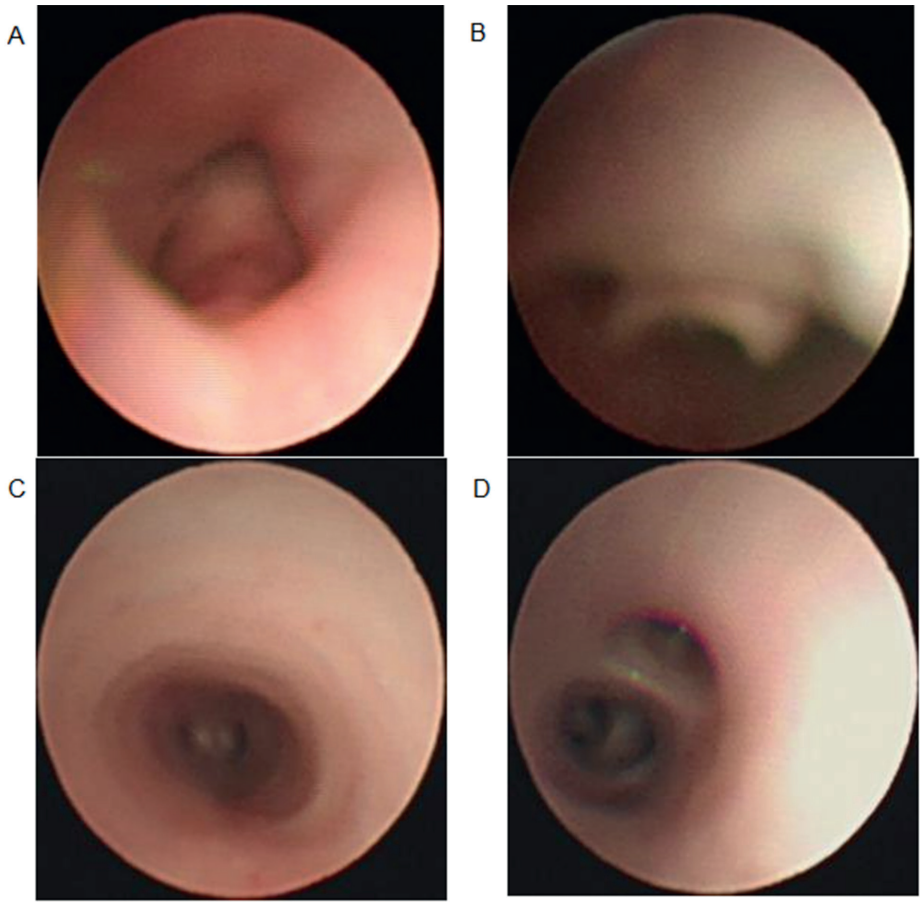

Figure 3. Fiberoptic bronchoscopy. **A**: Soft palate softening. **B**: Epiglottis softening. **C**:Tracheal carina softening. **D**: Bronchial endometritis.

chromosome 16p13.3 region were discovered in some RSTS patients, which confirmed that RSTS was an autosomal dominant genetic disease. It is mainly caused by the variation of homologous genes CREBBP and EP300[3-6]. The CREBBP gene is located at 16p13.3, contains 31 exons, and encodes a CREB binding protein containing 2442 amino acids. The EP300 gene is located at 22q13.2 and is 2415 amino acids in length[7]. The vast majority of cases are caused by gene mutations[8] and RSTS patients with a clear family history are very rare. About 55% of RSTS cases are caused by CREBBP gene abnormalities, and about 8% are related to homologous to CREBBP. Caused by the gene EP300, the etiology of the remaining cases is not yet clear[9]. Fergelot et al[10] conducted a comparative analysis on 52 children with EP300 gene mutation and 308 patients with CREBBP gene mutation and discovered that their clinical manifestations were similar. In patients with EP300 gene mutation, the most evident manifestation in kids is microcephaly. In addition, the incidence of pre-eclampsia in pregnant women with EP300 mutant fetuses is significantly higher than that of pregnant CREBBP fetuses[10]. The exon 13 of CREBBP gene in this patient had c.2368C>T heterozygous mutation. The 790th amino acid of the protein encoded by the CREBBP gene was mutated from glutamic acid to a stop codon (p.Q790*), and the 2368th cytosine was mutated to thymine. This mutation is a nonsense mutation, which leads to early termination of protein translation. It poses a significant impact on protein function, which leads to abnormal CREBBP function.

RSTS is a very rare disease caused by abnormal histone synthesis due to gene mutation. The disease was first reported by Rubinstein and Taybi in 1963[2]. In these patients, any organ can be affected, however, the leading manifestation is a lag in growth and development, intellectual disability, microcephaly, special face, wide thumb, thick first toe and eye disease. RSTS has corre-

sponding clinical manifestations at birth and with the growth, the disease gradually becomes more evident. The broad thumb and the big first toe are often characteristic signs of the disease, so it is also called the thumb-heel Toe syndrome. A small number of patients may have deformities, such as widened metatarsal bones and angled thumb[11]. Twenty-one percent of children with RSTS have growth retardation. These children are often born with normal length and weight. Due to feeding difficulties, laryngoplasty and recurrent respiratory tract infections along with other disorders, most of these children are prone to lag behind in length and weight. They were often lower than the 5th percentile of normal range[8]. The patient presented in this report was found to have a broad thumb, a wider first toe, and a right palm when he was admitted to the hospital for examination. The feeding difficulties were progressively worsen after the birth. The fiberoptic bronchoscopy showed symptoms of softening of the laryngocartilage and trachea. The baby had a smaller than normal jaw with a wry smile, he suffered from feeding difficulties and had a poor after birth weight gain and his facial features were relatively insufficient.

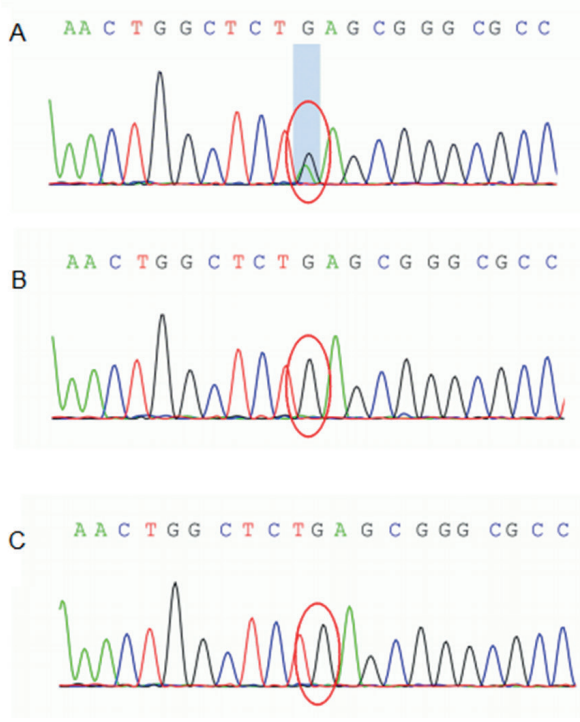

Figure 4. Genetic testing: there is a heterozygous mutation in the CREBBP gene. **A**: There is c.2368C>T mutation in the child. **B**: father has no mutation. **C**: mother has no mutation.

Neuropsychological developmental disorders are a common manifestation of RSTS. Typical RSTS in the neonatal period is characterized by low muscle tone, delayed mental and motor development, and varying degrees of intellectual disability. IQ values range from 25 to 79. 90% of children have delayed speech, and some children's IQ declines further with age[12-14]. There may be a link between the low IQ and autism characteristics of RSTS patients and a large number of gene deletions. RSTS has a higher incidence of secondary epilepsy, with an incidence of about 28%, and an abnormal EEG of about 60%[15,16]. In a study on 18 Chinese patients with RSTS, Yu et al[17] found 7 clinical characteristics including microcephaly, micrognathia, angled thumb, phalangeal deformity, polydactyly, autistic behavior, and epilepsy. There were significant differences compared with the Western RSTS population. Fourteen children received brain MRI examination, 57% of the children had abnormal brain structure and 29% had corpus callosum abnormalities. Corpus callosum abnormalities were the most common reported MRI abnormalities among RSTS patients. One of the children had a normal MRI report on the 48-day brain. A MRI review[17] at 14 months showed Chiari malformation type I, thinning of the corpus callosum, and hydrocephalus. In the present study, the brain MRI on 40 days after birth was normal but further follow-ups were considered. At the time of preparation of this report, patient was showing symptoms, such as low muscle tone, crying, and difficulty in appeasing the nerves. The neuropsychiatric development disorder was not prominent. Considering the fact that patient was diagnosed with this illness at a young age, some clinical symptoms needed further follow-up.

Possible malformations and complications in children with RSTS usually include: gastroesophageal reflux, constipation, congenital heart disease, renal malformations, cryptorchidism, ocular lesions, deafness, endocrine disorders, malignant tumors, etc[6]. The most prevalent types of eye lesions are cataracts, unilateral or bilateral iris/retinal/optic nerve deficiencies, lacrimal duct obstruction, refractive errors, strabismus and glaucoma[18-20]. Most children with RSTS are initially diagnosed in pediatrics, and eye diseases are often noticed because of abnormal appearance. Genderen et al[19] discovered 117 cases of eye abnormalities in 207 cases of RSTS, with 31 of them exhibited changes in congenital glaucoma. The most common ocular abnormalities found in the eyes of 24 RSTS patients were lacrimal duct

obstruction, corneal abnormalities, congenital glaucoma, congenital cataract, and keratoma. It was reported[19] that almost all fundus examinations demonstrated abnormalities, and retinal abnormalities were as high as 67%. In the study of 18 RSTS patients in China, Yu et al[17] reported that 38% of patients suffered from ocular abnormalities, including extraocular and intraocular abnormalities. The main manifestations were bilateral nasolacrimal duct obstruction, strabismus, bilateral trichiasis, etc. One patient with RSTS complicated with cytomegalovirus infection suffered from strabismus, corneal leukoplakia, glaucoma, nystagmus, iris abnormality[17]. There is a huge disparity among different reports studying the incidence of glaucoma in children with eye abnormalities. To our knowledge, there was only one case reported in China. The incidence rate in China is significantly different from that in the Western countries. In this report, we are presenting the youngest patient ever reported with abnormal eye diagnosed with glaucoma only 3 days after birth. Due to the extremely low incidence, this condition can be easily missed or misdiagnosed. In addition to congenital glaucoma, the child also suffered from strabismus, retinal dystrophy, and fundus lesions that require further examination to confirm the diagnosis. At the same time, more case studies are needed for children with cytomegalovirus infection, to further clarify whether there is correlation between RSTS glaucoma and cytomegalovirus infection.

More than 90% of patients with RSTS can live to adulthood, and the medical problems they face change with age[21]. A series of behaviors, such as anxiety, emotional instability, and combativeness appear in adolescence. The biggest problem of adult patients is overweight or obesity[22]. In 2003, Wiley et al[16] issued a guideline for diagnosis and treatment of RSTS, which explained in detail the management and treatment of various systems and the role of family and society in the treatment of this disease. They choose treatment based on clinical manifestations: nasal feeding, language training, heart surgery, glaucoma surgery, orthopedic surgery, prevention of gastroesophageal reflux and respiratory infections[16]. With the continuous understanding of the clinical manifestations and genetics knowledge of RSTS, in 2015, Milani et al[6] proposed that after the clinical diagnosis of RSTS, assessments were required in neuropsychiatric, hearing, vision, bone, cardiovascular, skin and endocrine system. MRI of the head and spinal cord, renal ultrasound and genetic testing were also considered essential. During puberty, all the above assessments and stress tests need to be repeated. At the age of 6 months, 1 year, 18 months, 2 years, 30 months, and after 3 years, the focus should be on hearing, vision, bones and teeth. At the time of this report, our patient has different degrees of damage to the eyes, cardiovascular, urinary system, feeding, respiratory tract and nervous system. The current treatment of this child is mainly to control the infection and strive to complete the glaucoma surgery as soon as possible. Nasal feeding improved nutrition condition, created conditions for rehabilitation training, and reduced the degree of developmental backwardness. Cardiac ultrasound, electroencephalogram, brain MRI, and hearing examination should be regularly reviewed to observe whether hearing damage and head structure changes may occur. At the same time, we should also pay attention to the weight of the children to prevent the rapid increase in body weight. One of the 18 patients reported by Yu et al[17] had obesity at 7 months and was misdiagnosed as Prader Willi syndrome. In addition, we should pay attention to identify the early manifestations of malignant tumors and intervene in time.

Literature[17] shows that most children with RSTS have been hospitalized in NICU due to severe conditions such as severe pneumonia, hypoglycemia, and sepsis. But the diagnosed age of the children varies from 2 months to 12 years old. Therefore, improving the diagnosis and identification of newborn RSTS is very important. Although RSTS syndrome is an autosomal dominant inheritance method, most patients have new mutations, so they are mostly sporadic and the risk of recurrence is about 0.5% to 1.0%[6]. There are also very few reports[23,24] of vertical transmission or continuous involvement of siblings, so it is still necessary to conduct genetic counseling and prenatal diagnosis before giving birth again. About genetic counseling, for parents with germline or chimeric mutations, genetic testing through blood or saliva is very important to assess the risk of recurrence.

Conclusions

In this paper, a patient with congenital glaucoma combined with respiratory tract infection in the neonatal period was examined using gene sequencing. The diagnosis of RSTS was clarified and the genetic testing and clinical manifestations of RSTS, especially eye lesions, were reviewed

in the literature. FGFR-related craniosynostosis syndrome (e.g., Pfeiffer syndrome, Apert syndrome) can also show thumb or thumb angle deformity. Therefore, early clinical diagnosis is very difficult. The genetic variation spectrum is an important basis for diagnosis. Generally, 55-70% of clinically diagnosed RSTS cases are diagnosed by genetic testing[3]. Patients with the same nonsense mutations can show evident phenotypic differences, and multi-center studies are still needed to expand our knowledge of clinical phenotypes. In this article, neonatal glaucoma was diagnosed as the first symptom, which provided basis for diagnosis of RSTS.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' Contributions

FH and AZ conceived and designed the study, and drafted the manuscript. FH, AZ, JX and LH collected, analyzed and interpreted the experimental data. AZ and LH revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Hunan Provincial People's Hospital. Signed written informed consents were obtained from the patient's guardians.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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