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Case study

Identification of a novel de novo mutation of *CREBBP* in a patient with Rubinstein-Taybi syndrome by targeted next-generation sequencing: a case report☆

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Summary Rubinstein-Taybi syndrome (RSTS) is a rare autosomal dominant congenital disorder (prevalence, 1:125 000-720 000) characterized by broad thumbs and halluces, facial dysmorphism, psychomotor development delay, skeletal defects, abnormalities in the posterior fossa, and short stature. The purpose of this study was to use targeted exome sequencing to identify the genetic cause of RSTS in a 6.5-year-old girl presenting typical features of this condition. Targeted exome sequencing of the patient DNA revealed de novo transition c.1066C>T corresponding to a novel nonsense mutation p.Q356X in the CREB-binding protein gene, *CREBBP*, whose haploinsufficiency is responsible for 50% to 60% of the RSTS cases. Based on comparing the clinical manifestations of our patient with those of patients carrying similar mutations, we supposed that haploinsufficiency is the possible functional consequence of p.Q356X mutation by creation of a loss-of-function *CREBBP* allele due to a premature stop codon and RSTS phenotype. Our findings expand the spectrum of mutations associated with this condition.

1. Introduction

Rubinstein-Taybi syndrome (RSTS) (OMIM no. 180849) is a congenital disorder characterized by mental and growth retardation and a wide range of typical dysmorphic features. Facial dysmorphology includes down-slanted palpebral fissures, a broad nasal bridge, a beaked nose, and micrognathia.

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Particularly noticeable are the broad thumbs and broad big toes [1]. In addition, patients with RSTS have an increased risk of tumor formation [2].

RSTS is autosomal dominant, but reports of transmission are rare, most cases being caused by de novo mutations [3]. The mutations found in patients vary from point mutations (nonsense, missense, frameshift, and splice site), to large microdeletions removing the entire gene, duplications, translocations, and inversions of chromosome 16p13.3 encompassing the critical RSTS gene for CREB-binding protein (*CREBBP*) [1,2,4-6].

The protein CREBBP (OMIM no. 600140) serves as a transcriptional coactivator. It has a transactivation domain but does not specifically bind to DNA. The name of the protein is based on the interaction with the CRE-binding protein (CREB); however, CREBBP interacts with a large number of other proteins as well. It is thought that CREBBP acts as an integrator of the signals from various pathways [4]. Transcription factors downstream from these pathways need to compete with each other for the limited amount of CREBBP available in the nucleus. The protein forms a physical bridge between the DNA-binding transcription factors and the RNA polymerase II complex. In addition, CREBBP has intrinsic histone acetyl transferase (HAT) activity. By acetylating histones, CREBBP opens the chromatin structure at the locus that needs to be expressed, a process essential for gene expression. CREBBP is also capable of acetylating a large number of other proteins—for example, the transcription factor p53 [4].

CREBBP shares homology with another protein, p300, which is encoded by the *EP300* gene (OMIM no. 602700, E1A-binding protein) on chromosome 22q13.2. Both proteins are particularly homologous at their binding sites for transcription factors, and p300 also has a HAT domain. Like CREBBP, it serves as a transcriptional coactivator. Mutations in *EP300* gene explain from 5% to 8% of RSTS patients [1,5]. *EP300* is, therefore, a gene candidate to be screened.

Several molecular techniques, such as fluorescence in situ hybridization [7], denaturing high-performance liquid chromatography [8], array comparative genome hybridization [9], DNA direct sequencing [10], real-time polymerase chain reaction [11], and multiplex ligation-dependent probe amplification [12], have been used to identify mutations in the *CREBBP* gene so far. We report on a child with multiple congenital anomalies and intellectual disability who had a novel *CREBBP* mutation and demonstrate that targeted exome sequencing (TES) of a disease-specific subset of genes, followed by criteria for prioritization of possibly pathogenic changes, is a reliable and efficient method for detecting point mutations of the *CREBBP* gene in well-characterized patients with RSTS features.

2. Materials and methods

2.1. Case presentation

A 6.5-year-old girl was admitted to our facility for evaluation of facial dysmorphism, broad thumbs and halluces, and mental and motor delay suggesting RSTS. There was no relevant family history. She was second in birth order of nonconsanguineous parents with an older brother without any developmental delay. Pregnancy was slightly complicated by flu-like infection during the first trimester but continued uneventfully up to a normal delivery. Baby was born at term and was small for gestational age; birth weight was 2850 g, and birth length was 49 cm. Congenital stridor was noted at birth, which is still occasionally present. Jaundice prolonged up to the end of the first month of life. The parents noticed some developmental delay at age of 6 months, which became obvious later on, when some coordination instability appeared. The girl experienced repeated airway infections in the second half of the first year, when skin allergy to cephalosporin and constantly increased leukocyte number (~18×10^9) even out of infection were registered. Optical return loss testing excluded hearing loss at age 14 months. Normal female karyotype and corpus callosum agenesis on magnetic resonance imaging have been proved at age 15 months. At age 24 months, she was unable to stand up or speak. She started walking at age 2 years, but the expressive speech was substantially delayed. Epileptic equivalents were suspected at age 3 years, but electroencephalogram proved to be normal. The parents noted a presence of abnormal behavior and almost constant nasal discharge of yellowish secretion.

At age 6.5 years, her weight was 23 kg (standard deviation score [SDS], −0.40), height was 110 cm (SDS, − 1.10), and head circumference was 49 cm (SDS, −1.16). Clinical evaluation showed distinctive phenotype: hypertelorism; slanting down; asymmetric palpebral fissures because of a mild left-sided ptosis; distinctive beaked nose with deviated and broad nasal bridge, extending below the nares; high arched and narrow palate; smaller mandible (Fig. 1A); and normal muscle tone with slightly increased reflexes. Her hands were small and broad with short and proximally implanted thumbs (Fig. 1B). Her feet were small with short and stubby first toes. Evaluation of cognitive functioning revealed moderate mental delay with IQ 48 and abnormal behavior, characterized by negative, at times aggressive, attitude to surrounding people. Brain magnetic resonance imaging showed low-degree lateral ventriculomegaly as a result of complete corpus callosum agenesis (data not shown). The patient had unremarkable blood and urine test results except the increased leukocyte count (14 × 10^9) (Granulocytes, 51.3%; Monocytes, 11.5%; Lymphocytes, 36.7%) and higher monocyte fraction level. Previous genetic analyses excluded microdeletions or duplications of chromosome 16p13.3 suspected to cause a phenotype consistent with RSTS.

2.2. Genetic analysis

Peripheral blood samples of the proband and parents were collected by venipuncture, and genomic DNA was extracted according to standard protocols [13].