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Genetics Corner: A New Case of Rubinstein-Taybi Syndrome with a Novel Variant in the CREBBP Gene Detected through Whole Exome Sequencing

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Abstract:

Rubinstein-Taybi syndrome (RSTS), an autosomal-dominant neurodevelopmental disorder affecting 1 in 125,000 newborns, is characterized by intellectual disability, growth retardation, facial dysmorphisms, and skeletal abnormalities. RSTS results from mutations in epigenetic machinery genes: CREBBP (~60%) or its homologous EP300 (~10%). Up to 30% of patients lack identified causative mutations, complicating early diagnosis due to phenotypic overlap with other syndromes. Here, we report a new RSTS case in an infant with atypical presentation. Whole-exome sequencing at 20 months revealed a de novo heterozygous pathogenic variant in CREBBP, c.6067C>T (p.Gln2023), establishing the diagnosis. This case introduces a new CREBBP gene variant, illustrating the broad clinical spectrum of Mendelian disorders of the epigenetic apparatus. High WES diagnostic rates emphasize its utility in cases with challenging phenotypes spanning distinct syndromes.*

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Introduction:

Rubinstein-Taybi syndrome (RSTS, OMIM 180849, 613684) is an exceptionally rare autosomal dominant genetic disorder, with an estimated prevalence of one case per 125,000 live births (1). Initially documented in 1963 by Rubinstein, a pediatrician, and Taybi, a radiologist, RSTS is a rare neurodevelopmental multisystem malformation syndrome characterized by developmental delay and intellectual disability (DD/ID), growth retardation, skeletal anomalies (such as broad/short thumbs and/or big toes), and distinctive facial features (e.g., downslanting palpebral fissures, broad nasal bridge, low hanging columella) (2). Individuals with RSTS may also exhibit a diverse range of anomalies and malformations, including cardiac and genitourinary abnormalities, recurrent infections, feeding difficulties, constipation, and hearing loss (3). Typically occurring sporadically, RSTS is linked to loss-of-function mutations in the homologous genes cAMP-response element binding protein (CREB)-binding protein (CREBBP) and EA1 binding protein p300 (EP300) in 50–75% of cases (4). The phenotypic overlap between RSTS and other Mendelian conditions often complicates the clinical diagnosis of RSTS (5). This report presents a newly identified case of RSTS with novel variants presented with an atypical clinical presentation, diagnosed through whole exome sequencing.

Case Description:

A 41-day-old female with a personal medical history of laryngomalacia and failure to thrive was admitted to LLUCH in January 2022 for acute respiratory failure. After admission, she was also found to have microtia, webbed toes, an aberrant right subclavian artery, and a patent ductus arteriosus. She was born full term at 39 weeks and 1-day gestation to a 39-year-old G3P2 mother via normal spontaneous delivery without complications. She passed her cardiac and hearing screens and was appropriate, though slightly small, for gestational age with a birth weight of 2.778 kg (15th %ile) and a birth length of 19.02" (33rd %ile). She had been conceived by *in vitro* fertilization. During the pregnancy, her mother did have a COVID-19 infection requiring hospitalization for a week. Otherwise, there were no other reports of preeclampsia, diabetes, or other exposures. Prenatal ultrasounds and screens were unremarkable. Both parents were healthy and had no physical abnormalities; the family history was not contributory.

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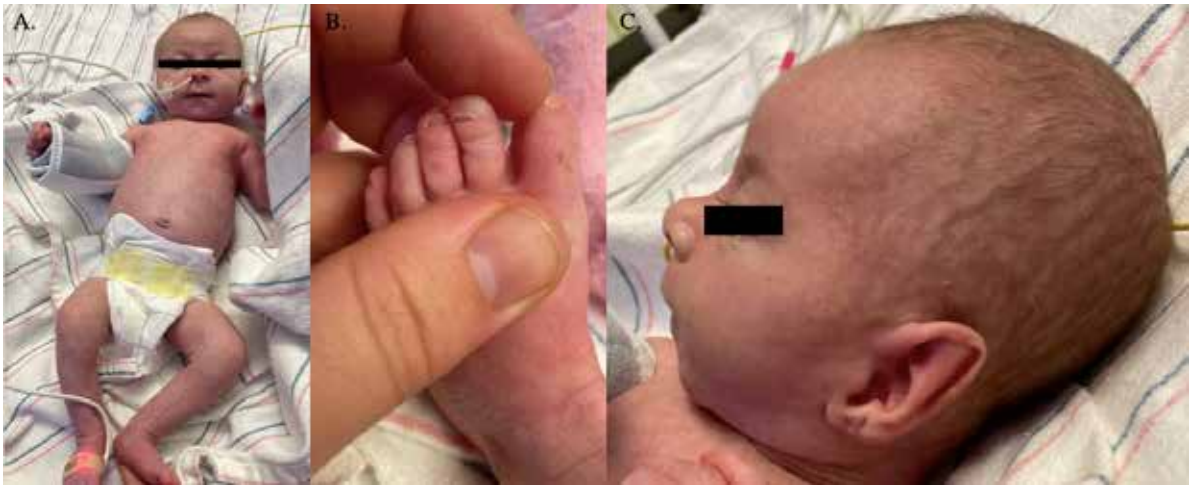
Figure 1: Patient's (A) full front profile (note the slight hypertelorism and broad and high nasal bridge), (B) webbed toes, and (C) uplifting earlobes with pits during her admission in January 2022, when she was 1.5 months old.



toes were noted lorism, a broad and high nasal bridge, uplifting earlobes with pits, and bilateral webbed toes were noted (Figure 1). Abdominal ultrasound was normal. A sleep study had found central sleep apnea. A head ultrasound found lenticulostriate vasculopathy of uncertain clinical significance and was otherwise normal. Given the heart defect and otherwise non-specific features, a chromosomal microarray was ordered and returned negative for chromosomal microdeletions and microduplications.

At eight months old, she returned to Genetics in the outpatient setting in August 2022. In between her discharge in January and this appointment, she had several admissions due to complications from her laryngomalacia, feeding intolerance, and Chiari malformation. Her suboccipital craniectomy the previous month was reported to improve her sleep apnea. She still had plagiocephaly and a bump on her forehead. At this stage, she was also noted to experience developmental delays. She was beginning to hold her head up and could not sit independently. She could roll over from her back to her stomach but not vice versa. Physical examination also noted epicanthal folds. Her parents consented to trio whole exome sequencing (WES); the blood was collected at 20 months old, which provided the diagnosis of Rubenstein-Taybi syndrome. He returned at 22 months old to discuss the WES result with her family. Her developmental delays persisted. She was still unable to walk or talk. She could stand with support at 18 months and sit independently at 14 or 15 months. She also lacked fine motor skills. As a result, she was a client of the Early Intervention program at the Inland Regional Center and received occupational and feeding therapy. New physical exam

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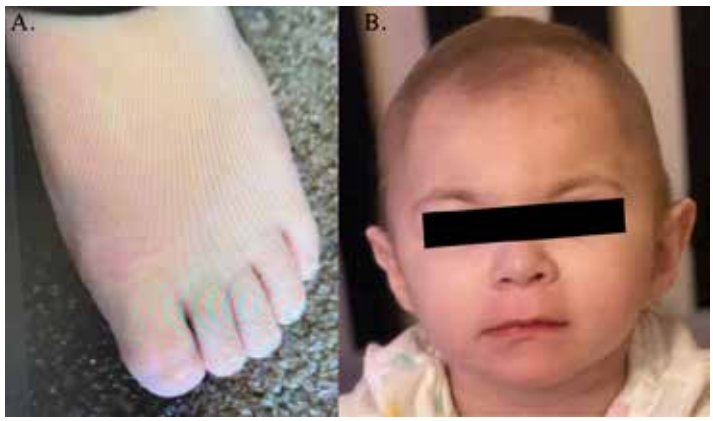
Figure 2: (A) Broad hallux and (B) low-hanging columella identified at 22 months old.



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findings included low-hanging columella, and broad halluces become apparent (Figure 2).

"Whole exome sequencing (WES) was performed at 20 months. WES identified a de novo heterozygous pathogenic variant in the CREBBP gene, c.6067C>T (p.Gln2023). This pathogenic variant has not been previously reported, is not listed in ClinVar, and is absent from control population databases. This variant occurs in the last exon of the CREBBP gene (exon 31 or 31 total exons)."*

Genetic Testing:

The initial chromosome microarray performed soon after birth was negative. Whole exome sequencing (WES) was performed at 20 months. WES identified a *de novo* heterozygous pathogenic variant in the *CREBBP* gene, c.6067C>T (p.Gln2023*). This pathogenic variant has not been previously reported, is not listed in ClinVar, and is absent from control population databases. This variant occurs in the last exon of the *CREBBP* gene (exon 31 or 31 total exons). It is predicted to cause premature protein truncation, though expected to escape nonsense-mediated decay due to its location in the last exon of the gene. It is predicted to delete approximately 18% of the protein, including the functional *CREB*-binding domain. Other truncating variants in this gene, including those further downstream of this variant, have also been associated with disease.

Discussion:

Here, we present a new case of Rubinstein-Taybi syndrome (RSTS) characterized by atypical manifestations during infancy (see Table 1 for the comparison of the typical features with our case). The conclusive diagnosis was established through whole exome sequencing conducted at 20 months of age. This underscores the challenging nature of RSTS diagnosis, given the considerable variability in phenotypes and genotypes, as

Table 1: the typical features of RSTS1 (4) and our patient's features.

Features of RSTS (incidence %)	Patient's clinical features
Typical facial features (100%)	Microtia, hypertelorism, broad and high nasal bridge, uplifting earlobes with pits, epicanthal folds, low-hanging columella
Intellectual disability (~100%)	Global developmental delay
Cryptorchidism (78–100%)	—
Microcephaly (35–94%)	Microcephaly
Broad thumbs/halluces (96%)	Broad halluces, webbed toes
Speech delay (90%)	Speech delay
Recurrent respiratory infections (75%)	—
Delayed bone age (74%)	—
Constipation (40–74%)	Constipation
Talon cusps (73%)	—
Gastroesophageal reflux (68%)	Gastroesophageal reflux disease
EEG abnormalities (57–66%)	—
Renal anomalies (52%)	—
Refractive defects, glaucoma, retinopathy (>50%)	—
Congenital heart defects (24–38%)	Aberrant right subclavian artery, patent ductus arteriosus
Seizures (25%)	—
Keloids (24%)	—
Deafness (24%)	—
Growth retardation (21%)	Initial failure to thrive
Malignant tumors (3–10%)	—
Spinal cord tethering (<5%)	—
Other	Sleep apnea, laryngomalacia, feeding intolerance, Chiari malformation, strabismus, astigmatism

documented by Spena et al. in 2015(3). Furthermore, our case highlights those phenotypic changes, particularly those emerging during growth, were only discerned through clinical re-evaluation prompted by the results of whole exome sequencing (5). This underscores the significance of whole exome sequencing as a reliable and expeditious diagnostic tool for suspected genetic diseases, as emphasized by Yu et al. in 2019 (6).

"The CREBBP gene is one of the most frequently reported genetic contributors to Rubinstein-Taybi syndrome (RSTS). Presently, approximately 500 pathogenic variants have been documented within CREBBP, constituting the identified etiology in 50–60% of all RSTS cases, whereas mutations in EP300 (OMIM 602700) account for only 5% of cases. The mutation spectrum encompasses diverse types, with 80.2% attributed to point mutations. Truncating mutations predominate among point mutations, comprising 55.2%, followed by large rearrangements (18.8%), missense mutations (16.8%), and splice mutations (9.2%). Notably, CREBBP lacks distinct hotspot mutation sites, with the mutation spectrum distributed across all 31 exons."

CREBBP Gene Mutations

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Disease pathogenesis:

The CREBBP gene, located on 16p13.2, and its counterpart, the E1A binding protein p300 (EP300), situated on 22q13.2, are integral players in fundamental cellular processes such as DNA repair, cell growth, differentiation, apoptosis, and tumor suppression. Acting as transcriptional co-activators across diverse signaling pathways, they significantly influence normal fetal development (7). Deletion or mutation of a single copy of the CREBBP gene leads to a consequential reduction in CREBBP protein production, compromising antenatal and postnatal developmental processes. Beyond developmental implications, CREBBP's involvement in multiple signaling pathways heightens RSTS patients' risk of developing both non-cancerous and cancerous tumors, including leukemia and lymphoma (10). This underscores the intricate interplay between genetic mutations and the potential for tumorigenesis in individuals affected by RSTS.

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Genotype and phenotype correlation:

Establishing a conclusive genotype–phenotype correlation in Rubinstein-Taybi syndrome (RSTS) has proven challenging (11). Recent studies, however, offer valuable insights into the roles of the CREBBP and EP300 genes in neural cell and brain development, particularly in regulating precursor cell migration and neuronal plasticity (12). RSTS is categorized into two types based on the associated mutation spectrum: RSTS1 (OMIM#180849), linked to the CREBBP mutation spectrum, and RSTS2 (OMIM#613684), associated with the EP300 mutation spectrum. The classic phenotype resulting from CREBBP gene deletions or truncating mutations manifests as intellectual disability, broad thumbs, and distinctive facial dysmorphism. Notably, the severity of symptoms is more pronounced in cases of CREBBP mutations compared

to EP300 mutations (13). A severe phenotype of RSTS1, known as the chromosome 16p 13.3 contiguous deletion syndrome, results from large deletions encompassing the CREBBP gene and adjacent 3' genes, including DNASE1 and TRAP1(14). This severe form often presents with profound mental retardation, life-threatening infections, systemic complications, and other classic features. Notably, pathogenic variants in CREBBP exons 30 and 31 have been associated with Menke-Hennekam syndrome (15). Although Menke-Hennekam shares similarities such as developmental delay, intellectual disability, feeding difficulties, autistic behavior, recurrent upper airway infections, hearing impairment, short stature, microcephaly, and facial dysmorphism, it differs from Rubenstein-Taybi in specific facial features and the absence of classic broad/angulated thumbs or halluces observed in RSTS patients. Despite these classifications, emerging evidence suggests a lack of significant correlation between phenotype and mutation type, location, or deletion size for either CREBBP or EP300 genes in RSTS patients (16). This underscores the intricate nature of the relationship between genotype and phenotype in the context of Rubinstein-Taybi syndrome.

Management:

The approach to managing individuals with Rubinstein-Taybi syndrome (RSTS) is tailored to the specific presenting abnormalities. With over 90% of affected individuals surviving into adulthood and achieving varying degrees of independence in self-care and communication, life expectancy is generally normal. However, it may be compromised in individuals with RSTS who are prone to infections or have severe congenital heart defects, underscoring the importance of promptly addressing these complications. Behavioral disorders, mood swings, and obsessive-compulsive disorders may emerge as individuals with RSTS transition to adulthood. Simultaneously, ongoing research explores therapeutic strategies targeting the molecular pathology of RSTS, with many interventions currently in the preclinical testing phase. Given the irreversible nature of most genetic mutations and the high reversibility of epigenetic modifications, therapeutic targeting and modulation of altered epigenetic components in RSTS present a promising avenue for future treatment modalities (4).

Conclusions:

Rubenstein-Taybi Syndrome represents a rare genetic disorder characterized by distinctive physical attributes, growth impediments, and intellectual disabilities. The diagnostic intricacy arises from shared clinical features with multiple syndromes and the dynamic phenotype evolution during growth, posing challenges for early detection. A pivotal tool in achieving diagnostic precision is whole exome sequencing. In this context, the identification of the genetic variant in the presented case contributes to the molecular elucidation of RSTS. This report enhances the collective understanding of RSTS and broadens the spectrum of genetic variants associated with the intricate complexity of the CREBBP gene in this disorder.

Practical Applications:

- Rubinstein–Taybi syndrome (RSTS) is an autosomal-dominant neurodevelopmental disease characterized by intellectual disability, growth retardation, facial dysmorphisms and skeletal abnormalities.
- The diagnostic intricacy arises from shared clinical features with multiple syndromes and the dynamic phenotype evolution during growth, posing challenges for early detection.
- Whole exome sequencing plays a critical role in confirming a definitive diagnosis.
- Managing individuals with Rubinstein-Taybi syndrome is tailored to the specific presenting abnormalities.

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