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ORIGINAL ARTICLE

Novel heterozygous variants in the *EP300* gene cause Rubinstein–Taybi syndrome 2: Reports from two Chinese children

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Abstract

Background: Rubinstein–Taybi syndrome (RSTS) is a rare autosomal-dominant genetic disease caused by variants of *CREBBP* (RSTS1) or *EP300* (RSTS2) gene. RSTS2 is much less common, with less than 200 reported cases worldwide to date. More reports are still needed to increase the understanding of its clinical manifestations and genetic characteristics.

Methods: The clinical data of two children with RSTS2 were analyzed retrospectively, and their clinical manifestations, auxiliary examinations, and mutational spectrum were summarized. Liquid chromatography–tandem mass spectrometer (LC–MS/MS) technology was used to detect the levels of steroid hormones if possible.

Results: After analyzing the clinical and genetic characteristics of two boys with RSTS2 (0.7 and 10.4 years old, respectively) admitted in our hospital, we identified two novel heterozygous variants in the *EP300* exon 22 (c.3750C > A, p. Cys1250*, pathogenic; c.1889A > G, p. Tyr630Cys, likely pathogenic), which could account for their phenotype. In addition to common clinical manifestations such as special facial features, microcephaly, growth retardation, intellectual disability, speech delay, congenital heart defect, recurrent respiratory infections, and immunodeficiency, we found one of them had a rare feature of adrenal insufficiency, and LC–MS/MS detection showed an overall decrease in steroid hormones.

Conclusion: In our study, we identified two novel variants in the *EP300* exon 22, and for the first time, we reported a case of RSTS2 associated with adrenal insufficiency, which will enrich the clinical and mutational spectrum of this syndrome.

KEYWORDS

adrenal insufficiency, *EP300*, genotype, LC–MS/MS, Rubinstein–Taybi syndrome

Caiqi Du, Zhuoguang Li and Biao Zou authors contributed equally to this work and share first authorship.

1 | INTRODUCTION

Rubinstein–Taybi syndrome (RSTS) is a rare autosomal-dominant genetic disorder first reported by Rubinstein and Taybi in 1963 (Rubinstein & Taybi, 1963). RSTS has an estimated incidence of 1:100,000 to 1:125,000 (Milani et al., 2015; Van Gils et al., 2021) and is known to be caused by variants of the *CREBBP* (RSTS1, OMIM #180849) (Petrij et al., 1995) and *EP300* (RSTS2, OMIM #613684), which is a CREBBP homolog (Zimmermann et al., 2007). In general, only about 55%~70% clinically diagnosed RSTS patients can be confirmed by genetic testing (Milani et al., 2015). Among them, RSTS2 caused by *EP300* accounts for 8%~10%, and less than 200 cases have been reported worldwide. RSTS is primarily characterized by special facial features, broad thumbs and halluces, growth retardation, and mild to severe intellectual disability. Other findings may include recurrent respiratory infections, immunodeficiency, congenital heart defects, renal malformations, and endocrine disorders (such as congenital hypothyroidism, thyroid hypoplasia, and growth hormone deficiency) (Korzus, 2017; Saettini et al., 2020; Spena et al., 2015). RSTS2 appears to be associated with a milder phenotype, they may have less severe facial dysmorphism and better cognitive function compared to those with RSTS1 (Milani et al., 2015).

Currently, little is known about genotype–phenotype correlations of RSTS and there is no literature linking RSTS to the occurrence of adrenal insufficiency. Here, we report two novel heterozygous variants in the *EP300* exon 22 (c.3750C > A, p. Cys1250*; c.1889A > G, p. Tyr630Cys) and describe a patient associated with adrenal insufficiency among our two cases of RSTS, which will enrich the clinical and mutational spectrum of RSTS.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

This study was approved by Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Approval number: TJ-IRB20180703). Written informed consent was provided from their parents.

2.2 | Subjects

The data of two patients with RSTS2 diagnosed by genetic testing in the Department of Pediatrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology were analyzed retrospectively.

2.3 | Whole-exome sequencing

After obtaining the informed consents, whole-exome sequencing analysis (AmCare Genomics Lab, China) was performed on the probands and their parents. Target genes included 5177 genes collected from the OMIM database with definite corresponding diseases. The sequencing covered coding exons, and known pathogenic variants reported in deep introns or noncoding regions were also included. The average coverage depth was about 200 with over 99.6% of the target regions covered by at least 20 reads. Sequenced reads were compared with the reference human genome version (GRCh37/hg19). Sequence variants were annotated using population and literature databases including 1000 Genomes, dbSNP, GnomAD, Clinvar, HGMD, and OMIM. Some online software was used to analyze the structure of the protein, predict the conservation domain, function domain, and perform the multiple sequence alignment. Variants were interpreted according to the ACMG guidelines (Richards et al., 2015).

3 | RESULTS

3.1 | Patient 1

The proband, an 8 months old boy, was admitted to the hospital due to cough with wheezing for 6 days (seen in Table 1). He was born at 39 week gestation to a G1P1 mother via cesarean section delivery as a small for gestational age (SGA) neonate, with body weight 2250 g (<3rd centile) and length 45.0 cm (<3rd centile). He was fed with formula milk after birth and received complementary foods at 7 months old. He could hold his head up at 4 months and sit steadily at 7 months old.

During the neonatal period, he was diagnosed of hypothyroidism and has been treated with oral L-Thyroxine tablets until now. He was hospitalized several times for serious respiratory infection after birth. His mother had a history of gestational hypertension. His parents were from two unrelated families with no family history of inherited or metabolic diseases. His father is 178 cm tall (75th~90th centile) and his mother is 166 cm tall (75th~90th centile).

3.1.1 | Physical examinations

His height was 67.5 cm (3rd~10th centile), weight was 8.0 kg (10th~25th centile), and occipital frontal circumference (OFC) was 42.2 cm (<3rd centile). The anterior fontanel was nearly closed. He had low-set ears and suffered hearing impairment in his right ear. Rib valgus was found bilaterally in the thorax. The areola and nipple was

TABLE 1 Clinical baseline data and genetic features of the two patients with RSTS.

Characteristics	Patient 1	Patient 2
Gender	Male	Male
Age at diagnosis (year)	0.7	10.4
Height (cm)	67.5	135.5
Height (centile)	3rd~10th	10th~25th
Weight (kg)	8.0	35.7
Weight (centile)	10th~25th	50th~75th
Chief complaint	Cough with wheezing	Fever
Concomitant symptoms	Small for gestational age; Microcephaly; Low-set ears; Right ear hearing impairment; Recurrent respiratory infections; Immunodeficiency; Patent ductus arteriosus; Hypothyroidism; Adrenal insufficiency; Mild mental retardation	Recurrent respiratory infections; Neutropenia; Immunodeficiency; Speech delay; Mild mental retardation
Genotypes		
Base change	c.3750C > A	c.1889A > G
Amino change	p. Cys1250*	p. Tyr630Cys
Exon	22	22
Source	De novo	De novo

darkly pigmented, and the scrotum was slightly dark. The volume of both the testes was less than 1 mL. Moist rales can be heard in the lungs on auscultation. The liver was palpable 2 cm below the right rib, but the spleen was not palpable.

3.1.2 | Auxiliary examinations

At admission, there were no obvious abnormalities in liver function, kidney function, serum electrolytes, serum lipid, or respiratory pathogen examinations. The complete blood count suggested infection (white blood cell count 28.36×10^9/L, neutrophil 44%, lymphocyte 39.8%, monocyte 6.8%, and eosinophil 9.2%). Immune function: IgA < 0.07↓g/L (0.11~1.45 g/L) and IgM 0.21↓g/L (0.33~1.75 g/L) were lower than normal levels. Thyroid function implied hypothyroidism. Adrenocortical hormones (chemiluminescence immunoassay): adrenocorticotropic hormone (ACTH) < 5.00 pg/mL, cortisol (F) 78.33 nmol/L, 17-hydroxyprogesterone (17-OHP) 1.41 nmol/L, androstenedione (A4) < 0.01 nmol/L, dehydroepiandrosterone sulfate (DHEA-S) < 1.00 nmol/L, and testosterone (T) < 0.01 nmol/L. Chest CT scan showed infection in bilateral lungs. Ultrasonic cardiogram revealed patent ductus arteriosus (1.4 mm) and a left-to-right interatrial shunt (2.8 mm).

3.1.3 | Follow-up results

After the diagnosis, the patient received oral hydrocortisone replacement therapy and other relevant treatments, and was followed up every 3 months. When he was 13 months old, detection of serum steroid hormones using LC–MS/MS revealed a significant decrease of adrenocortical hormones, mineralocorticoids (11-deoxycorticosterone, corticosterone), glucocorticoids (11-deoxycortisol, F, cortisone), progesterone (17-hydroxypregnenolone, 17-OHP), and androgens (DHEA, A4) levels. The result was shown in Figure 1 and Supplementary Materials. At the last follow-up visit (4 years old), his intelligence assessment suggested that he was half a year behind his peers. And the comparison of steroid hormones during treatment was shown in Supplementary Materials. His immune function was still significantly lower than normal range (IgA < 0.07↓g/L, IgG 1.5↓g/L, and IgM 0.20↓g/L) and gamma globulin therapy was recommended.

3.2 | Patient 2

The proband, a 10.4 years old boy, was admitted with fever for 6 days (seen in Table 1). He was born to a G3P3 mother with a full-term pregnancy by natural vaginal delivery. He was fed with breast milk after birth and received complementary foods at 1 year old. He could walk at 1 year old, but he had speech delay and did not speak until 3 years old. His parents were from two unrelated families with no family history of inherited or metabolic diseases. His father was 173 cm tall (50th~75th centile) and his mother was 148 cm tall (< 3th centile).

3.2.1 | Physical examinations

His height was 135.5 cm (10th~25th centile) and weight was 35.7 kg (50th~75th centile). Slightly broad thumbs and halluces were seen. Left and right testes were 2 mL (left) and 3 mL (right), respectively. Other organs such as heart, lung, abdomen, and nervous system showed no obvious abnormalities.

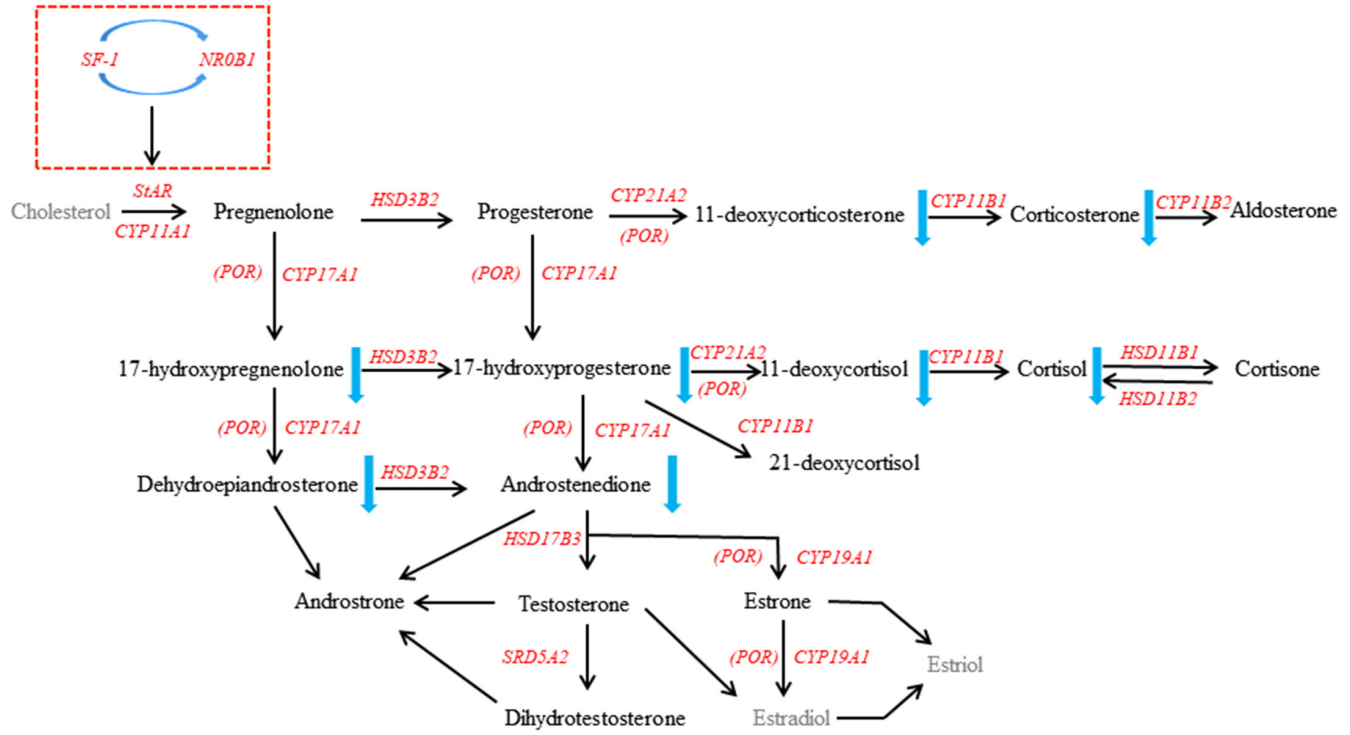

FIGURE 1 The trend of steroid hormone changes in patient 1 (LC–MS/MS technology, the arrow shows the trend of hormone changes).

3.2.2 | Auxiliary examinations

At admission, the complete blood count suggested agranulocytosis (white blood cell count 1.62×10^9/L, neutrophil count 0.09×10^9/L, neutrophil 5.4%, lymphocyte 59.3%, and monocyte 27.8%). Inflammatory indicators: CRP: 68.77 mg/L, ESR 59 mm/h, and Ferritin: 594.6 μg/L. The levels of immune function (IgG, IgA, IgM, and complement C3 and C4), thyroid function, and adrenocortical hormones (ACTH, F, 17-OHP, A4, DHEA-S and T) were in the normal range. Anti-EchoVirus IgM and anti-CoxsackieVirus Group B IgM were positive, but no abnormalities were found in other etiology and culture tests. Intelligence assessment suggested mild intellectual disability. Due to financial reasons, his parents declined LC–MS/MS for steroid hormone testing, so this part of the data was missing.

3.3 | Genetic testing results

In both Patient 1 and 2, we identified novel heterozygous variants in the *EP300* exon 22 (patient 1: c.3750C > A, p. Cys1250*; patient 2: c.1889A > G, p. Tyr630Cys), which were confirmed by Sanger sequencing. There was no such variant at this site in their parents, so the variants could be considered as de novo variants. See Table 1 and Figure 2 for details.

As for the c.3750C > A (p. Cys1250*) variant, according to the 2015 ACMG guidelines, this variant belongs to PVS1 (nonsense variant), PS2 (de novo variant, both maternity and paternity confirmed, no family history, and parent–child relationship confirmed), and PM2 (low frequency mutation in the normal population database). Therefore, this frameshift variant is a likely pathogenic variant, which could account for the phenotype of the proband.

As for the c.1889A > G (p. Tyr630Cys) variant, according to the 2015 ACMG guidelines, this variant belongs to PS2 (de novo variant, both maternity and paternity confirmed, no family history, and parent–child relationship confirmed), PM2 (low frequency mutation in the normal population database), and PP3 (multiple lines of computational evidence support a deleterious effect on the gene or gene product). Therefore, this missense variant is a likely pathogenic variant, which could account for the phenotype of the proband.

4 | DISCUSSION

RSTS is a rare autosomal-dominant genetic disease caused by variants of *CREBBP* (RSTS1) or *EP300* (RSTS2) gene. Current studies have demonstrated that the phenotype of the RSTS patients is heterogeneous (Korzus, 2017; Saettini et al., 2020; Spena et al., 2015). As a multisystem disorder, RSTS has been reported with many clinical manifestations, including dysmorphic facial features, growth and psychomotor developmental delays, recurrent respiratory infections,

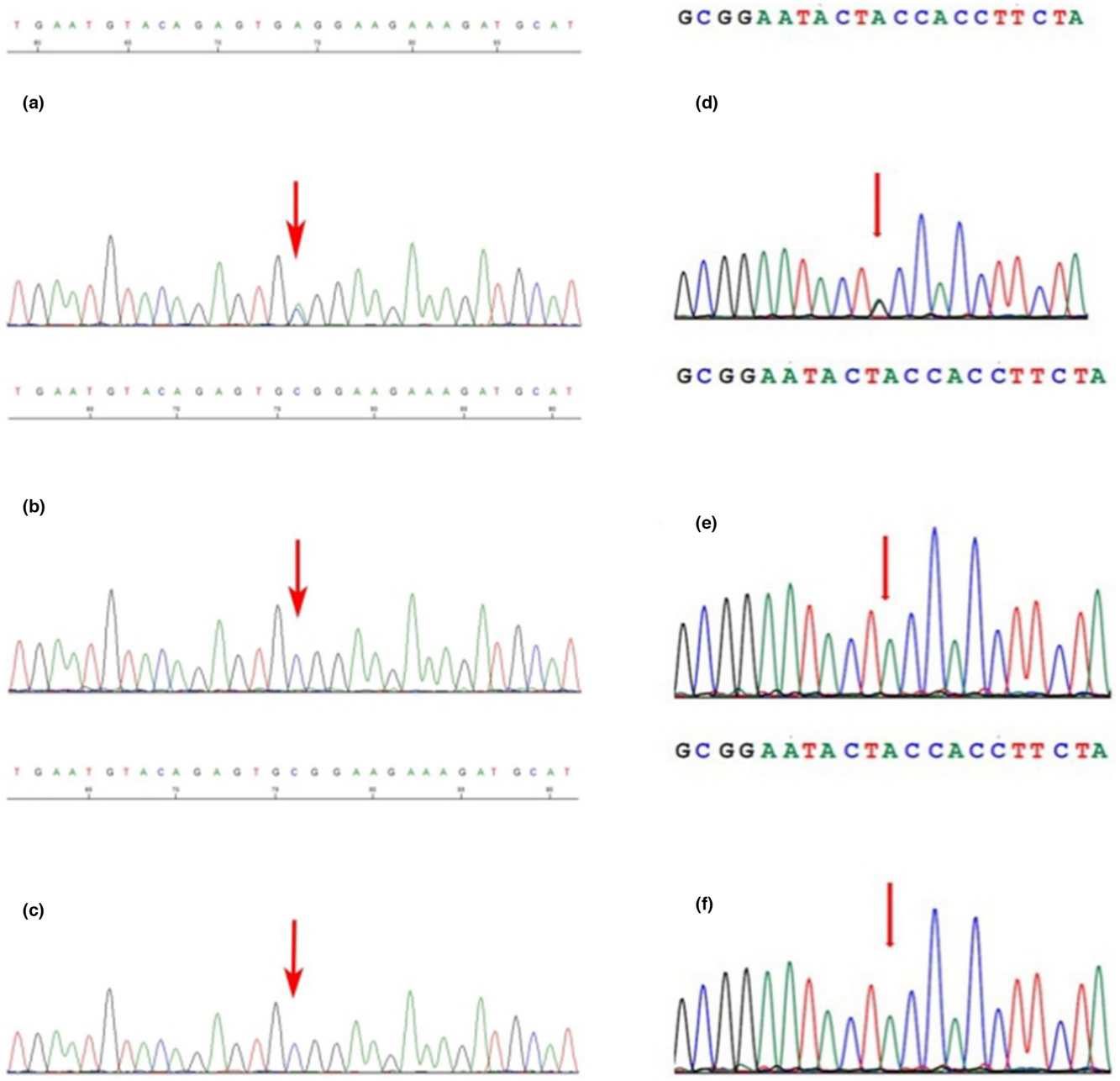

FIGURE 2 The genetic test results of the two RSTS2 patients and their parents (Genome reference sequence: GRCh37/hg19). Patient 1: (a): a heterozygous variant of EP300 in the proband (c.3750C > A, p. Cys1250*). (b): no variant of EP300 in the proband's father. (c): no variant of EP300 in the proband's mother. Patient 2: (d): a heterozygous variant of EP300 in the proband (c.1889A > G, p. Tyr630Cys). (e): no variant of EP300 in the proband's father. (f): no variant of EP300 in the proband's mother.

immunodeficiency, congenital heart defects, renal malformations, and endocrine disorders. However, the presence of adrenal insufficiency in RSTS patients has never been reported to date. As for patient 1, he was admitted not only with recurrent respiratory infections, but he also suffered from immunodeficiency, intellectual disability, congenital heart defect, and hypothyroidism. He had microcephaly and hearing impairment in the right ear, but he did not show the typical facial features as *CREBBP* caused RSTS1 such as grimacing smile and short broad thumbs. It's worth our attention that the adrenal insufficiency in this patient was a new finding different from previous reports, which we would discuss below. As for patient 2, his clinical manifestations were mild. He was also hospitalized with an infection, and he had mild intellectual disability and broad thumbs (halluces). Even with normal immunoglobulin levels, immunodeficiency should be considered because of his history of recurrent infections and neutropenia or even agranulocytosis. Although immunodeficiency is not a

typical symptom of RSTS2, the c.1889A > G variant of *EP300* gene in our patient is located in the KIX domain, some studies believe that this functional domain can mediate the interaction between *EP300* and *c-Myb* gene, thereby will play an important role in hematopoiesis (Kasper et al., 2013).

The *EP300* gene, encoding a protein, p300, is located on chromosome 22q13. *EP300* variant in RSTS was first reported in 2007 (Zimmermann et al., 2007), and most, if not all, mutations occur de novo. According to HGMDPro databases, 175 pathogenic variants of *EP300* gene have been reported, including missense/nonsense variants (42.2%), splicing variants (5.7%), small deletions or insertions variants (39.4%), small indels variants (2.3%), and gross deletions variants (9.7%). After genetic analysis, we identified two novel heterozygous variants in the *EP300* exon 22 (c.3750C > A, p. Cys1250*; c.1889A > G, p. Tyr630Cys), which were classified as pathogenic (PVS1 + PS2 + PM2) or likely pathogenic (PS2 + PM2 + PP3) variants according to ACMG guidelines. During steroid hormone biosynthesis, the rate-limiting and regulatable step is the transport of cholesterol from the outer to the inner mitochondrial membrane, a process mediated by the steroidogenic acute regulatory (StAR) protein (Manna et al., 2003). Regulation of the human *CYP11A* gene encoding cytochrome P450scc, which catalyzes the conversion of cholesterol to pregnenolone and is the first step of steroid hormone biosynthesis, is regulated by many trans-acting transcription factors including steroidogenic factor 1 (SF-1) (Monté et al., 1998). SF-1 is critical for steroid gene expression and plays an important role in the development, differentiation, and function of multiple endocrinal organs, including the hypothalamus, pituitary, adrenal glands, and gonads (Parker & Schimmer, 1997). CBP/p300, a factor known to interact with multiple transcription factors, is proved to be involved in CREB/SF1-mediated regulation of *StAR* gene transcription (Manna et al., 2003). CBP/p300-interacting transactivator with ED-rich tail 2 (CITED2) binds *EP300* and *CREBBP*, and in mice lacking *Cited2*, embryos will die from adrenal agenesis, cardiac malformations, abnormal cranial ganglia, and exencephaly (Bamforth et al., 2001). Several reports have demonstrated that p300 plays an important role in regulating the function of SF-1 (Chen et al., 2005). In addition, SF-1 can promote the expression of P450scc (*CYP11A1*) promoter in adrenal cortex Y1 cell, and TReP-132 is involved in the regulation of P450scc gene expression. It is speculated that these genes coordinately regulate the expression of P450scc, and the regulation of P450scc gene by SF-1 is mediated by p300/CBP (Monté et al., 1998).

In our study, we also analyzed the patient's serum steroid hormone profile using LC–MS/MS technology. Compared with immunoassay methods, LC–MS/MS can more comprehensively and truly reflect the levels of steroid hormones. Through changes in the levels of various steroid hormones, we can judge the abnormality of the steroid pathway more intuitively (Dahl et al., 2018; Li et al., 2021). The overall decrease in steroid hormones of patient 1 suggested that his anabolic disturbance may be related to upstream pathway blockage. Initially, the possibility of lipoid congenital adrenal hyperplasia (LCAH) associated with StAR deficiency was also suspected clinically. However, no adrenal-related gene abnormalities were found in whole-exome sequencing analysis. Therefore, we further explored whether there was a correlation between *EP300* gene and steroid pathway regulatory genes such as *StAR*. Based on the above literature review, we hypothesized that the patient's adrenal insufficiency may be associated with *EP300* variant. But why this situation does not exist in other *EP300* caused RSTS2 is also a point that confuses us. Here, we raise the idea that RSTS2 may be associated with adrenal insufficiency, and we hope that be more evidence from clinical reports and basic studies will answer this question in the future.

5 | CONCLUSION

In conclusion, among our two rare cases of RSTS2, we identified two novel heterozygous variants in the *EP300* exon 22 (c.3750C > A, p. Cys1250*; c.1889A > G, p. Tyr630Cys) and found that one patient was accompanied with adrenal insufficiency. This is the first published report of adrenal insufficiency associated with RSTS2. We believe these additional reports will help us enrich the clinical and mutational spectrum of RSTS.

AUTHOR CONTRIBUTIONS

XPL and SNS conceptualized and designed the study. CQD, ZGL, and BZ supervised data collection, reviewed the analyses, and wrote all versions of the article. XSL, FC, and YL coordinated and supervised data collection, critically reviewed the article, and approved the final article as submitted. All authors approved the final article as submitted and agree to be responsible for all aspects of the work.

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None.

CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

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REFERENCES

Bamforth, S. D., Bragança, J., Eloranta, J. J., Murdoch, J. N., Marques, F. I., Kranc, K. R., Farza, H., Henderson, D. J., Hurst, H. C., & Bhattacharya, S. (2001). Cardiac malformations, adrenal agenesis, neural crest defects and exencephaly in mice lacking Cited2, a new Tfap2 co-activator. *Nature Genetics*, *29*(4), 469–474. https://doi.org/10.1038/ng768

Chen, W. Y., Juan, L. J., & Chung, B. C. (2005). SF-1 (nuclear receptor 5A1) activity is activated by cyclic AMP via p300-mediated recruitment to active foci, acetylation, and increased DNA binding. *Molecular and Cellular Biology*, *25*(23), 10442–10453. https://doi.org/10.1128/mcb.25.23.10442-10453.2005

Dahl, S. R., Nermoen, I., Brønstad, I., Husebye, E. S., Løvås, K., & Thorsby, P. M. (2018). Assay of steroids by liquid chromatography-tandem mass spectrometry in monitoring 21-hydroxylase deficiency. *Endocrine Connections*, *7*(12), 1542–1550. https://doi.org/10.1530/ec-18-0453

Kasper, L. H., Fukuyama, T., Lerach, S., Chang, Y., Xu, W., Wu, S., Boyd, K. L., & Brindle, P. K. (2013). Genetic interaction between mutations in c-Myb and the KIX domains of CBP and p300 affects multiple blood cell lineages and influences both gene activation and repression. *PLoS ONE*, *8*(12), e82684. https://doi.org/10.1371/journal.pone.0082684

Korzus, E. (2017). Rubinstein–Taybi syndrome and epigenetic alterations. *Advances in Experimental Medicine and Biology*, *978*, 39–62. https://doi.org/10.1007/978-3-319-53889-1_3

Li, Z., Liang, Y., Du, C., Yu, X., Hou, L., Wu, W., Ying, Y., & Luo, X. (2021). Clinical applications of genetic analysis and liquid chromatography tandem-mass spectrometry in rare types of congenital adrenal hyperplasia. *BMC Endocrine Disorders*, *21*(1), 237. https://doi.org/10.1186/s12902-021-00901-8

Manna, P. R., Wang, X. J., & Stocco, D. M. (2003). Involvement of multiple transcription factors in the regulation of steroidogenic acute regulatory protein gene expression. *Steroids*, *68*(14), 1125–1134. https://doi.org/10.1016/j.steroids.2003.07.009

Milani, D., Manzoni, F. M., Pezzani, L., Ajmone, P., Gervasini, C., Menni, F., & Esposito, S. (2015). Rubinstein–Taybi syndrome: Clinical features, genetic basis, diagnosis, and management. *Italian Journal of Pediatrics*, *41*, 4. https://doi.org/10.1186/s13052-015-0110-1

Monté, D., DeWitte, F., & Hum, D. W. (1998). Regulation of the human P450scc gene by steroidogenic factor 1 is mediated by CBP/p300. *The Journal of Biological Chemistry*, *273*(8), 4585–4591. https://doi.org/10.1074/jbc.273.8.4585

Parker, K. L., & Schimmer, B. P. (1997). Steroidogenic factor 1: A key determinant of endocrine development and function. *Endocrine Reviews*, *18*(3), 361–377. https://doi.org/10.1210/edrv.18.3.0301

Petrij, F., Giles, R. H., Dauwerse, H. G., Saris, J. J., Hennekam, R. C., Masuno, M., Tommerup, N., van Ommen, G. J., Goodman, R. H., Peters, D. J., & Breuning, M. H. (1995). Rubinstein–Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature*, *376*(6538), 348–351. https://doi.org/10.1038/376348a0

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., & Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Rubinstein, J. H., & Taybi, H. (1963). Broad thumbs and toes and facial abnormalities. A possible mental retardation syndrome. *American Journal of Diseases of Children*, *105*, 588–608. https://doi.org/10.1001/archpedi.1963.02080040590010

Saettini, F., Herriot, R., Prada, E., Nizon, M., Zama, D., Marzollo, A., Romaniouk, I., Lougaris, V., Cortesi, M., Morreale, A., Kosaki, R., Cardinale, F., Ricci, S., Domínguez-Garrido, E., Montin, D., Vincent, M., Milani, D., Biondi, A., Gervasini, C., & Badolato, R. (2020). Prevalence of immunological defects in a cohort of 97 Rubinstein–Taybi syndrome patients. *Journal of Clinical Immunology*, *40*(6), 851–860. https://doi.org/10.1007/s10875-020-00808-4

Spena, S., Gervasini, C., & Milani, D. (2015). Ultra-rare syndromes: The example of Rubinstein–Taybi syndrome. *Journal of Pediatric Genetics*, *4*(3), 177–186. https://doi.org/10.1055/s-0035-1564571

Van Gils, J., Magdinier, F., Fergelot, P., & Lacombe, D. (2021). Rubinstein–Taybi syndrome: A model of epigenetic disorder. *Genes (Basel)*, *12*(7), 968. https://doi.org/10.3390/genes12070968

Zimmermann, N., Acosta, A. M., Kohlhase, J., & Bartsch, O. (2007). Confirmation of EP300 gene mutations as a rare cause of Rubinstein–Taybi syndrome. *European Journal of Human Genetics*, *15*(8), 837–842. https://doi.org/10.1038/sj.ejhg.5201791

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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