Rubinstein–Taybi because of a novel EP300 mutation with novel clinical findings

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Introduction

Rubinstein–Taybi syndrome (RSTS) is a rare congenital syndrome with an incidence of 1/100 000 to 1/125 000 of live births, with more than 90% of patients surviving into adulthood (Blum, 1995; Hennekam, 2006; Milani et al., 2015a). Although this syndrome has an autosomal dominant pattern of inheritance, the majority of described cases have been attributed to de novo mutations found in one of two genes: CREBBP (cAMP-response element binding protein) on chromosome 16p13.3 or EP300 (E1A-binding protein) on chromosome 22q13.2 (Rubinstein and Taybi, 1963). Approximately 50–60% of cases are caused by mutations in CREBBP [Rubinstein–Taybi syndrome type 1 (RSTS-1)], 10% are caused by mutations in EP300 [Rubinstein–Taybi syndrome type 2 (RSTS-2)], and 30% are of unknown etiology (Bartsch et al., 2005; Hennekam, 2006; Zimmermann et al., 2007; Negri et al., 2016). CREBBP and EP300 share regions of high sequence similarity and encode histone acetyltransferases that bind the cAMP-response element-binding protein, a gene known to influence cell proliferation and differentiation and tumor suppression (Bartholdi et al., 2007).

The hallmark features of RSTS include broad and angulated thumbs and halluces, intellectual disabilities, delayed developmental milestones, postnatal growth retardation, and dysmorphic facial characteristics including downslanting palpebral fissures, a prominent nose with a low columella, a thin upper lip, and a pouting lower lip (Bartholdi et al., 2007). Other common features include cryptorchidism, dental anomalies such as talon cusps or malocclusions, hirsutism, congenital heart defects, poor feeding in early life with esophageal reflux, and adolescent obesity (Stevens and Bhakta, 1995; Milani et al., 2015b; Negri et al., 2016). Despite many similarities, phenotypic variations between RSTS-1 and RSTS-2 patients have been described. Intellectual disabilities are less severe in patients with EP300 mutations; however, many of these individuals have neuropsychiatric issues such as anxiety and various behavioral problems (Milani et al., 2015b; Negri et al., 2016). Interestingly, maternal gestational hypertension and pre-eclampsia have been linked to nearly half of RSTS-2 cases (Solomon et al., 2015; Milani et al., 2015b; Negri et al., 2016). Patients with RSTS-2 have a greater propensity for craniofacial malformations such as microcephaly, but show better postnatal growth (Bartsch et al., 2010). Notable skin changes such as pilomatrixomas, keloids, and nevi are common in RSTS-2, but skeletal malformations of the hands and feet and radial deviation of the thumbs typical of RSTS-1 are often mild or altogether absent in RSTS-2 patients (Stevens and Bhakta, 1995; Bayle et al., 2004; Bartholdi et al., 2007; Bartsch et al., 2010; Negri et al., 2016).

To date, 23 confirmed cases of RSTS because of EP300 mutations have been reported (Roelfsema et al., 2005; Bartholdi et al., 2007; Zimmermann et al., 2007; Foley et al., 2009; Bartsch et al., 2010; Tsai et al., 2011; Bounakis et al., 2016)

Key features

- Microcephaly
- Down-slaning of palpebral fissures
- Thin upper lip
- Deviation of the nasal septum
- Prominent beaked nose
- Shallow philtrum
- High-arched palate
- Low-set earlobes
- Posteriorly rotated ears
- Hypoplasia of the labia majora
- Long eyelashes with prominent eyebrows
- Low frontal and occipital hairline
- Hirsutism
- Renal calcifications
- Hypoplastic aorta/coarctation/stenosis/anomaly/aortic arch interruption
- Broad/halluces
- Feeding disorder/dysphagia/swallowing/sucking disorder/esophageal dyskinesia
- Failure to thrive/difficulties for feeding in infancy/growth delay

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et al., 2015; Masuda et al., 2015; Negri et al., 2015, 2016; Solomon et al., 2015). Of these 23 cases, only one patient has presented with multiple congenital anomalies (Solomon et al., 2015). We report on a female patient with a novel de novo EP300 mutation with multiple congenital anomalies.

**Clinical report**

The patient is the first child of healthy, non-consanguineous parents (mother, age 30; father, age 32). The family history was unremarkable and the pregnancy was uneventful. Delivery occurred vaginally at 35 weeks of gestation with no complications. Weight at birth was 2070 g (10–25th percentile), length was 46 cm (25–50th percentile), and head circumference measured 30 cm (3–10th percentile). Apgar scores were 5 and 6 after 1 and 5 min, respectively. The patient presented with gastroesophageal reflux, feeding difficulties, and stridor, and was referred to the neonatal ICU because of respiratory distress.

Microcephaly, a low frontal and occipital hairline, a prominent beaked nose, down-slanting of palpebral fissures, thin upper lip, deviation of the nasal septum, shallow philtrum, mild micrognathia, high-arched palate, low-set earlobes, and posteriorly rotated ears were noted on physical exam (Fig. 1). The patient also had long eyelashes with prominent eyebrows and generalized hirsutism. Halluces were broadened bilaterally, but no additional bone abnormalities were noted (Fig. 2). The patient also showed hypoplasia of the labia majora. No additional skin changes were observed. Electroencephalography, otoacoustic emission, and brain MRI were normal. Echocardiographic examination showed a right-sided aortic arch with an aberrant left subclavian artery arising from the retroesophageal diverticulum. The presence of an aortic vascular ring was confirmed by computed tomography angiography (Fig. 3). The vascular ring was surgically repaired and the stridor was completely resolved after extubation.

Abdominal ultrasound showed normal adrenal glands with renal medullary hyperechogenicity (RMH) (Fig. 4). In addition, the patient presented with persistent, asymptomatic, normovolemic hyponatremia varying between 118 and 124 mmol/l and mild hyperkalemia varying between 4.4 and 6.3 mmol/l. Serum and urine concentrations of cortisol, aldosterone, and 17-OH progesterone were normal. Urinary excretion of potassium, calcium, and phosphorus was normal and sodium excretion varied between 21 and 74 mmol/l. Serum glucose levels were normal and no glycosuria was present.

At 7 weeks, the patient’s weight had increased to 3420 g (25–50th percentile), length was 57 cm (<10th percentile), and head circumference measured 34 cm (<10th percentile). The patient was discharged from the neonatal unit after 9 weeks and followed up by endocrinological and nephrological specialists. At the time of discharge, the patient continued to have feeding difficulties and showed poor weight gain.
At 12 months of age, she showed further growth retardation [length 72 cm (3–10th percentile), weight 7.3 kg (3–10th percentile)], and microcephaly [head circumference 40 cm (< 3rd percentile)]. She also had persistent RMH and treatment-resistant hyponatremia. Motor milestones and cognitive development milestones were also delayed. The patient was able to sit upright only with assistance and has not shown language development.

Human studies and informed consent: written informed consent was obtained from the patient’s parents.

**Methods and results**

**Cytogenetic and molecular analysis**

A karyotype analysis showed 46,XX. Multiplex ligation-dependent probe amplification for microdeletion/microduplications syndromes and DNA microarray were normal. Blood samples of the patient and parents were sent to the Department of Clinical Genetics, Institute of Mother and Child in Warsaw, Poland, for whole-genome array and whole-exome sequencing analysis. Array comparative genomic hybridization was performed on DNA extracted from peripheral blood cells (Chemagic Prepito, Prepito DNA Cyto Pure Kit; PerkinElmer Waltham, Massachusetts, USA) of the patient using commercially available arrays (CytoSure, ISCA 8×60 K v2.0; Oxford Gene Technology, Oxfordshire, UK) according to the manufacturer’s protocol. The arrays did not show pathogenic changes. The exome capture was performed using the NimbleGen SeqCap EZ HGSC VCRome 2.1 kit (NimbleGen, Pleasanton, California, USA). Over 16 Gbp of Illumina paired-end sequencing (2×100 bp) data (Illumina, San Daigo, California, USA) were generated, which resulted in a 94× mean coverage, with 93% of the bases covering at least 20×. Fastq data were mapped against the human reference genome (hg19) with Burrows-Wheeler Aligner (version 0.7.8) ([http://bio-bwa.sourceforge.net/](http://bio-bwa.sourceforge.net/)) and variant calling was performed using the GATK HaplotypeCaller (version 3.2.2) (Broad Institute, Cambridge, Massachusetts, USA). The coverage statistics were calculated with Picard (version 1.118) (Broad Institute, Cambridge, Massachusetts, USA) and Annovar (version 11-0882013) (Annovar, Redwood City, California, USA) was used for variant annotation.


**Discussion**

RSTS-2 is a rare genetic condition caused by a de novo mutation of the EP300 gene that presents with a diverse range of phenotypes. Almost all patients with RSTS-2 have only a single major congenital anomaly in addition to the characteristic features shown in RSTS. The most commonly observed anomalies are of the spine or the genitourinary system, found in 29% of patients. Other findings include anomalies of the cardiovascular system in 21%, skeletal anomalies (excluding the spine) in 12.5%, brain anomalies in 8%, and kidney anomalies in 8% (Roelfsema et al., 2005; Bartholdi et al., 2007; Zimmermann et al., 2007; Foley et al., 2009; Bartsch et al., 2010; Tsai et al., 2011; Bounakis et al., 2015; Negri et al., 2015, 2016; Solomon et al., 2015). In the current literature, there is only one previously described case of an RSTS-2 patient with multiple congenital anomalies.
(Solomon et al., 2015). Our case adds a further report of a patient with multiple abnormalities.

The clinical characteristics of our patient are consistent with previously described RSTS (Rubinstein and Taybi, 1963; Negri et al., 2016). Alongside these, we noted several novel anomalies including a vascular ring formed by a right-sided aortic arch and an aberrant left subclavian artery.

Renal abnormalities occur frequently in patients with RSTS-1 (50% of cases), and yet have only been observed in two cases of RSTS-2 (8%). In RSTS-1, the most common renal anomalies include hydronephrosis, collecting system duplications, vesicoureteral reflux, renal stones, and nephrotic syndrome (Chen, 2006). To date, none of these anomalies have been described in RSTS-2 patients, although renal agenesis was described in one case and renal lobation was discovered during an autopsy of a patient with an EP300 mutation, but not clinically diagnosed RSTS-2 (Woods et al., 2014; Solomon et al., 2015).

We describe a novel renal anomaly in a RSTS-2 patient, persistent RMH. In healthy neonates, RMH is a common self-limiting condition that resolves within 10–14 days postpartum (Howlett et al., 1997). Although the etiology of RMH remains poorly understood, the primary cause in neonates is associated with the accumulation of an endogenous substance that leads to tubular obstruction (Hemachandar and Boopathy, 2015). As such, many neonates present with nephrocalcinosis that is often accompanied by hypercalciuria and hyperuricemia. Other causes of RMH may be iatrogenic, commonly occurring as a side effect of furosemide therapy or because of renal injury or malformations (Slovis et al., 1993; Nakamura et al., 1999; Makhoul et al., 2005). Our patient showed no evidence of perinatal renal injury, maintained normal kidney function, and was normocalciuric, which suggested the persistent RMH to be the result of a structural congenital anomaly (Howlett et al., 1997).

Further electrolyte testing showed moderate hyponatremia and mild hyperkalemia, an electrolyte combination attributed most often to hypoadosteronism, owing to acute or chronic adrenal insufficiency (Peddle et al., 2008). Supplementary laboratory examination showed that the patient was normovolemic with normal serum levels of cortisol, aldosterone, and 17-OH progesterone and urinary steroid profile analysis. Adrenal causes of electrolyte imbalances were further ruled out when additional examination showed no evidence of infection and the patient was unresponsive to hydrocortisone and fludrocortisone. The combination of hyperkalemia, hyponatremia, normocalciuria, and normovolemia in infancy may also be suggestive of primary pseudo-hypoadosteronism, a mutation altering the number or function of aldosterone receptors; however, this potential diagnosis was excluded by whole-genome sequencing (Chang et al., 1996).

The cardiovascular system is another commonly affected system in RSTS patients of all etiologies, with abnormalities present in 33–35% of cases (Cantani and Gaglilesi, 1998; Lin and Ardinger, 2005). In RSTS-1, the most frequent cardiac malformations include patent ductus arteriosus, atrial septal defect, ventricular septal defect, and left-sided obstructions, such as coarctation of the aorta and hypoplastic left heart (Steven and Bhakta, 1995; Hanauer et al., 2002; Lin and Ardinger, 2005). Of the 23 cases of RSTS-2, only five cardiac abnormalities were reported, which included ventricular septal defect, patent ductus arteriosus, and an asymmetric aortic valve (Bartholdi et al., 2007; Zimmermann et al., 2007; Bartsch et al., 2010; Masuda et al., 2015; Negri et al., 2015). Our patient presented with a vascular ring formed by a right aortic arch and an aberrant left subclavian artery (lusoria artery) arising from the retroesophageal diverticulum. To our knowledge, these findings have not been described previously. Cardiac malformations, such as aortic arch remodeling, and left-right patterning abnormalities, such as right-sided aorta, have been recently linked to functional defects of a cotranscriptional factor, CITED2 (Bamforth et al., 2001; Sperling et al., 2005). This research carried out in mice showed that the interaction of CITED2 with EP300 and CREBBP leads to inhibition of histone acetyltransferases activity, which disrupts normal heart development (Bamforth et al., 2001; Sperling et al., 2005). Therefore, functional defects of EP300 and CREBBP associated with RSTS may play a similar role in promoting cardiac abnormalities in these patients.

The relationship between maternal pre-eclampsia and RSTS-2 has been of interest. Although pre-eclampsia was not present in our case, it has been reported in 10 (42%) cases (Bartholdi et al., 2007; Foley et al., 2009; Tsai et al., 2011; Negri et al., 2015, 2016; Solomon et al., 2015). In comparison, only one mother of an RSTS-1 child was reported to have maternal hypertension during pregnancy (Wieczorek et al., 2009). In a study looking at the Dutch population, there was evidence of linkage of chromosome 22 with pre-eclampsia; however, there was no such evidence for chromosome 16 (Lachmeijer et al., 2001).

**Conclusion**
We present the first case of a female RSTS-2 patient with persistent RMH, treatment-resistant hyponatremia, a right-sided aorta, and an aortic vascular ring. These novel congenital anomalies are offered to expand the phenotypic spectrum of disease and to further describe RSTS-2 as a disorder that can present with multiple major congenital abnormalities.
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Conflicts of interest

There are no conflicts of interest.

References


