

Rubinstein–Taybi syndrome 2 with cerebellar abnormality and neural tube defect

Kinga Hadzsiev^a, Zsuzsanna Gyorsok^b, Agnes Till^a, Márta Czakó^a and Oliver Bartsch^c

Rubinstein–Taybi syndrome (RSTS) is a rare dominant disorder with intellectual disability, postnatal growth deficiency, and multiple congenital anomalies. Approximately 50–70% of the patients have a mutation in the *CREBBP* gene (RSTS1) and 5–10% display an *EP300* gene mutation (RSTS2). Craniospinal abnormalities such as microcranium, scoliosis, and lordosis are frequent findings in RSTS1, but malformations of the brain or spinal cord are seen only occasionally. Here, we report on a 3-year-old boy with facial abnormalities of RSTS, broad thumbs and halluces, developmental delay, autistic features, cerebellar underdevelopment, and a neural tube defect. Molecular diagnostic of the *CREBBP* and *EP300* genes showed a heterozygous 17-bp deletion (c.5698_5714del AAGGCAGCAGGCCAGGT) in exon 31 of the *EP300* gene. Findings underline that small (hypoplastic) cerebellum and neural tube defects belong to the phenotypic spectrum not only of RSTS1 but also of RSTS2. Based on the literature and this observation, we recommend that each individual with RSTS2 should

be closely evaluated for neural axis and craniovertebral junction anomalies, and where appropriate, neuroimaging studies should be considered. Our frequency estimate of ~6% occult or overt neural tube defects in RSTS2 could represent an underestimate. *Clin Dysmorphol* 28:137–141 Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

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^aDepartment of Medical Genetics, Medical School, Clinical Center, University of Pécs, Pécs, ^bDepartment of Neurology, Bethesda Children Hospital, Budapest, Hungary and ^cInstitute of Human Genetics, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany

Correspondence to Kinga Hadzsiev, MD, PhD, Department of Medical Genetics, Clinical Center, University of Pécs, Szegedi ut 12, H-7624 Pécs, Hungary
Tel: +36 72 535 976; fax: +36 72 536 427; e-mail: hadzsiev.kinga@pte.hu

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Introduction

Rubinstein–Taybi syndrome (RSTS) is a rare autosomal dominant disorder with intellectual disability, postnatal growth deficiency, and multiple congenital anomalies. Rubinstein and Taybi (1963) described the characteristic dysmorphic features as follows: highly arched eyebrows, long eyelashes, down-slanting palpebral fissures, beaked nose with columella below alae nasi, high palate, grimacing smile, and broad thumbs, and halluces. Hennekam (2006) reviewed the clinical signs. The incidence of the syndrome is ~1: 100 000–125 000 (Beets *et al.*, 2014). There have been extremely rare observations of vertical transmission (Bartsch *et al.*, 2010), but the vast majority of RSTS cases occur sporadically because of a de novo mutation. Petrij *et al.* (1995) demonstrated the loss of one functional copy of the *CREBBP* gene in patients with RSTS. In 2005, *EP300* mutations were detected in patients with a clinical diagnosis of RSTS (Roelfsema *et al.*, 2005). Both *CREBBP* and *EP300* act as transcriptional coactivators in the regulation of gene expression

throughout different transduction pathways. Because a direct link between loss of acetyltransferase activity and RSTS could be observed and both genes (*CREBBP* and *EP300*) are histone acetyltransferases, it appears that aberrant chromatin regulation is the cause of the syndrome. Approximately 50–70% of patients showed a mutation in the *CREBBP* gene on chromosome 16p13.3 and have the disorder RSTS1 (OMIM 180849), and ~5–10% of the patients display a mutation of the *EP300* gene on chromosome 22q13.2 and have slightly different condition of RSTS2 (OMIM 613684). In ~30% of the patients, no mutation can be found using DNA studies from blood lymphocytes (Bartsch *et al.*, 2005; Hennekam, 2006).

Craniospinal abnormalities such as microcranium, scoliosis, and lordosis are frequent findings in RSTS, but malformations of the brain or spinal cord are seen rarely. In 2005, five patients were reported with a cervical myelopathy as the consequence of a stenosis at the craniovertebral junction (Yamamoto *et al.*, 2005). Soon thereafter, eight patients with RSTS were reported with tethered spinal cord and characteristic clinical manifestations including weakness of the lower extremities, leg and back pain, and neurogenic bladder, and the authors concluded that early detection and treatment of spinal defects in RSTS

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is important to prevent further neurologic disability (Tanaka *et al.*, 2006). Other cervical abnormalities associated with RSTS included Chiari malformation and syrinx in identical twin sisters, and the authors hypothesized that mesodermal growth insufficiency led to structural abnormalities of the face and basiocciput, including posterior fossa restriction, hindbrain herniation, Chiari malformation, and syrinx formation (Parsley *et al.*, 2011). It is also to be found in the literature that for neural axis and craniovertebral junction anomalies, full neuroimaging studies including brain and spine MRI and computed tomography scan of the cervicovertebral junction are advocated for patients with RSTS. Although the topic concerning neuroimaging is somewhat controversial, the authors suggest that it enables proper treatment and prevents neurologic deficits (Giusanni *et al.*, 2012). In their recent highly comprehensive update on clinical findings in 52 individuals with *EP300* mutations, Fergelot *et al.* (2016) reported one case each with a syringomyelia and a sacral lipomyelocele. They pointed out that information on the phenotypes and genotypes in *EP300*-mutated patients is still limited, but that only angulation of thumbs and halluces is extremely uncommon in these individuals, and that in general, clinical characteristics are qualitatively very similar between *CREBBP*-mutated and *EP300*-mutated patients, although some signs and symptoms are present at different frequencies. Furthermore, Fergelot *et al.* (2016) described that missense variants in parts of exon 30 and 31 of *CREBBP* and *EP300* can cause a phenotype not resembling RSTS1 or RSTS2, and recently, this new phenotype was delineated in more detail (Menke *et al.*, 2018).

Here we report on a 3-year-old boy with facial abnormalities of RSTS, broad thumbs and halluces, developmental delay, autistic features, cerebellar underdevelopment (inferior vermian hypoplasia), neural tube defect (lumbosacral myelocele, tethered cord, and syringohydromyelia), and a 17-bp deletion in exon 31 of the *EP300* gene (predicting a frameshift and, after 167 altered amino acid residues, a stop codon). Cerebellar abnormalities and neural tube defects have been reported occasionally with RSTS1/2, and here we report a combination of these abnormalities in another *EP300*-mutated patient.

Clinical report

The proband (Fig. 1) is the first child of healthy, nonconsanguineous parents (mother's age 42 years and father's age 59 years). Family history was unremarkable. Amniocentesis was performed because of advanced maternal age. Results were normal. In pregnancy week 36, the mother was hospitalized owing to pre-eclampsia, and he was born by cesarean section. Birth weight was 2470 g (10th to 25th percentile), length 49 cm (50th to 75th percentile), and head circumference 30.5 cm (10th percentile). On physical examination, a lumbosacral lipomyelocele was noted. An MRI of the brain and spinal cord showed hypoplasia

of the inferior portion of the cerebellar vermis, tethered cord, and mild syringohydromyelia. At the age 5 months, a rescue neurosurgical intervention (partial lipoma removal) was performed. Urological follow-up after neurosurgery disclosed an elevated sphincter tone, elevated bladder pressure, and grade IV vesicoureteral reflux on the right requiring vesicostomy at age 17 months. Neurological follow-up indicated global developmental delay. At the age 20 months, the proband was referred for genetic evaluation. He had microcephaly (43 cm, <3rd percentile), asymmetric skull, flat occipital region, low hairline, low-set dysplastic ears, arched eyebrows, long eyelashes, hypertelorism, down-slanting palpebral fissures, columella below alae nasi, moderately broadened thumbs and halluces, thoracic scoliosis, a well-healed surgical scar, and the vesicostoma. Neurological evaluation indicated esotropia, muscular hypotonia of the upper and lower extremities, increased deep-tendon reflexes in the lower limbs, and delayed motor and cognitive development. When last seen at age 2.5 years, he also showed delayed speech and autistic behaviors including stereotypic movements.

Methods

Array CGH and next-generation sequencing analysis were performed on DNA from peripheral blood cells of the patient. DNA was extracted using the DNA Purification Kit NucleoSpin Dx Blood (Macherey-Nagel, Düren, Germany) according to the protocol of the manufacturer. Array analysis was performed with the Agilent Human Genome G3 Sureprint 8×60K Microarray (Agilent Technologies, Santa Clara, California, USA), a high-resolution oligonucleotide-based microarray containing 55 077 60-mer probes. Labeling, hybridization, and washing were carried out according to the manufacturer's protocols (v7.2). Images were acquired with an Agilent G2565CA laser scanner, and results were obtained using the Agilent Feature Extraction (v11.5) and Cytogenomics (v2.9.2.4) software. Array CGH findings were normal.

For next-generation sequencing, we used a target enrichment procedure for all coding exons (including flanking intron sequences; ±20 bp) of the *CREBBP* and *EP300* genes with the SureSelectQXT Kit according to the manufacturer's protocols (Agilent Technologies). Sequence analysis was performed on a NextSeq 500 System (Illumina, San Diego, California, USA) using a MID-Output-Kit with the '2×150bp paired-end' sequencing chemistry (Illumina). The average coverage was 300 reads per amplicon. Read mapping and variant calling was carried out with the NextGENe Software (SoftGenetics, State College, Pennsylvania, USA) and the human reference genome (NCBI build GRCh38, release 106). Analysis of the *CREBBP* and *EP300* genes showed a heterozygous 17-bp deletion, c.5698_5714del AAGGCAGCAGGCCAGGT, in exon 31 (the last coding exon) of the *EP300* gene. The sequence alteration was confirmed by Sanger sequencing using a CEQ 8000

Fig. 1



The proband aged 20 months. (a, b) Facial views showing flat occipital region, low hairline, low-set dysplastic ears, arched eyebrows, long eye-lashes, hypertelorism, down-slanting palpebral fissures, and columella below alae nasi. (c) Left hand, note moderately broadened thumbs. (d) Right foot, note moderately broadened hallux.

Genetic Analysis System (Beckman Coulter, Krefeld, Germany) and the Mutation Surveyor V3.23 software (SoftGenetics), and predicted a frameshift and stop mutation (p.Lys1900Aspfs*167) following the CH3 domain, and thus likely a loss of the glutamine-rich and the IRF-3-binding domains of the EP300 protein (according to fig. 2 in Fergelot *et al.*, 2016).

The parents were not available for the genetic study to examine whether the mutation had occurred *de novo*. They consented to genetic testing of their son but not of themselves. However, both parents had normal phenotypes and notably no phenotypic features of RSTS2.

Discussion

RSTS1 and RSTS2 are caused by inactivating mutations of *CREBBP* and *EP300*, respectively, which are homologous genes encoding histone acetyltransferases (*CREBBP* and *p300*, respectively) and regulating transcription by

influencing the chromatin remodeling. RSTS1 has been extensively studied and is known to have a broad phenotypic spectrum. However, our knowledge of the phenotypic spectrum of RSTS2 is still limited, even with clinical data from ~70 *EP300*-mutated patients (Fergelot *et al.*, 2016). Angulation of thumbs and/or halluces is regarded a hallmark of only RSTS1 and is extremely uncommon in RSTS2, but in general, *CREBBP*-mutated and *EP300*-mutated patients share a very similar spectrum of clinical signs, although the individual signs occur with different frequencies (Fergelot *et al.*, 2016). Intellectual disability, postnatal growth retardation, and facial dysmorphism appear to be more pronounced in RSTS1, whereas microcephaly and behavioral problems have been observed more frequently in RSTS2.

Cerebellar abnormalities and neural tube defects have been reported as occasional abnormalities in RSTS1, and this report focuses on these findings in RSTS2. Clinical

findings in the proband included hypoplasia of the inferior portion of the cerebellar vermis, tethered cord, mild syringohydromyelia, and lumbosacral lipomyelocele. Similar but milder findings had been noted in previous *EP300*-mutated patients (Fergelot *et al.*, 2016). Patient 5 in that study showed syringomyelia (with a c.3857A>G, p.Asn1286Ser missense mutation), patient 17 had spina bifida occulta (and a c.256C>T, p.Arg86* stop mutation), and patient 25 showed a sacral lipoid myelomeningocele (c.4933C>T, p.Arg1645*) (Fergelot *et al.*, 2016). Patient 51 (with a c.4774A>T, p.1592* stop mutation) had a large cisterna magna, which could possibly show a cerebellar underdevelopment, but cerebellar hypoplasia was not reported in that patient; mega cisterna magna is present in 1–3% of the population, and is usually considered a benign variant of development. Hence, the incidence of neural tube defects in RSTS2 may be estimated at 3/52 (5.7%) or at 4/69 (5.8%) of patients, including this report and the 68 patients overseen by Fergelot *et al.* (2016). The true incidence could be even higher because not all patients with RSTS are routinely evaluated using brain and spine MRI, and milder neural tube defects (such as spina bifida occulta) may be missed in these patients.

High-grade vesicoureteral reflux as in this patient has been previously reported in *EP300*-mutated patients. Urogenital abnormalities including hydronephrosis, collecting system duplications, vesicoureteral reflux, and nephrotic syndrome were described in 50% of patients with RSTS1 (Chen, 2006) but in only 24% (11 of 45) of patients with RSTS2 (Fergelot *et al.*, 2016). In a previous report on RSTS with tethered spinal cord, four of eight probands had a neurological bladder and one proband had a high-grade vesicoureteral reflux (Tanaka *et al.*, 2006). Hence, we would suggest that some of the functional urinary tract abnormalities, such as the vesicoureteral reflux in this patient, could result from innervation defects caused by underlying neural tube defects (e.g. in this patient, the lipomyelocele).

Vermian hypoplasia is a rare malformation that may be caused by neurodevelopmental as well as metabolic defects, and malformations found to be associated with vermian hypoplasia have included migration abnormalities, agenesis of corpus callosum, and meningoencephalocoles (Cotes *et al.*, 2015). The syringomyelia in this patient could possibly be a consequence of a Chiari II malformation, as discussed elsewhere (Parsley *et al.*, 2011; Cotes *et al.*, 2015). There have been reports on Chiari I malformations in RSTS1, and some authors have reported a strong association between Chiari I malformations and cervicovertebral junction abnormalities (Giusanni *et al.*, 2012). However, we are not aware of reports on Chiari II malformations in *EP300*-mutated patients. A lack of closure of the neural tube leading to a leakage of fluid from the embryonic ventricular system and thus to a distension of the posterior fossa are widely

accepted as the cause of a Chiari II malformation in neuroectodermal disorders.

EP300-associated facial dysmorphisms may include long eyelashes in 90% of patients, microcephaly in 86%, arched eyebrows in 65%, down-slanting palpebral fissures in 56%, and low-set ears in 27% (Fergelot *et al.*, 2016). All of these features were present in our patient. Flat occiput and low posterior hairline have not been reported previously, but these are less relevant anomalies seen in numerous disorders. The intellectual disability is mostly mild in *EP300*-mutated patients, but behavioral problems such as autistic features and stereotypic movements have been reported in ~90%. Although this patient is still young, stereotypic movements, speech delay, and abnormal play activity were noted in his behavior as possible harbingers of an autism-spectrum disorder.

Maternal pre-eclampsia, alone or associated with intra-uterine growth retardation, has been reported in many *EP300*-mutated patients, including this proband. Decreased CREBBP/EP300 levels may impair the placenta's capacity to respond to low oxygen, resulting in pre-eclampsia (Van Uiter *et al.*, 2015). There is convincing evidence that pre-eclampsia occurs more frequently in RSTS2 than in the average pregnant population (2–10%), or in RSTS1 (3%) (Fergelot *et al.*, 2016; Jagla *et al.*, 2017). This could possibly be explained by the different expression profiles of CREBBP and EP300, correspondingly. CREBBP appears to be expressed continuously from the first trimester onward, whereas EP300 is detectable in placenta only in the later stages of pregnancy.

This report adds evidence that *CREBBP*-mutated and *EP300*-mutated patients share a very similar spectrum of clinical abnormalities, but the different signs manifest in different frequencies. Findings underline that neural tube defects and underdeveloped cerebellum belong to the phenotypic spectrum not only of RSTS1 but also of RSTS2. Our frequency estimate of some 6% occult or overt neural tube defects in RSTS2 is likely to represent an underestimate, because occasionally the symptoms may be unspecific and mild. Conceivably, the diagnosis of a mild neural tube defect could be missed, although it is important for the child and family in order to optimize treatment and avoid neurologic sequelae. On the basis of previous reports and this report, we suggest that each individual with RSTS2 should be closely evaluated for neural axis and craniovertebral junction anomalies by clinical specialists (pediatrician, child neurologist, clinical geneticist, pediatric radiologist, and neurosurgeon). Neuroimaging studies (e.g. brain and spine MRI) may be required in some cases, as has been similarly previously recommended for RSTS1 (Giusanni *et al.*, 2012).

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Conflicts of interest

There are no conflicts of interest.

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