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ARTICLE

Confirmation of *EP300* gene mutations as a rare cause of Rubinstein–Taybi syndrome

Nicole Zimmermann[1], Ana Maria Bravo Ferrer Acosta[2], Jürgen Kohlhase[2] and Oliver Bartsch*[,1]

[1]*Institute for Human Genetics, Johannes Gutenberg-University, Mainz, Germany;* [2]*Institute for Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany*

The Rubinstein–Taybi syndrome (RSTS, MIM 180849), a dominant Mendelian disorder with typical face, short stature, skeletal abnormalities, and mental retardation, is usually caused by heterozygous mutations of the *CREBBP* gene, but recently, *EP300* gene mutations were reported in three individuals. Using quantitative PCR (for the *CREBBP* and *EP300* genes) and genomic sequencing (for the *EP300* gene), we studied here 13 patients who had shown no mutation after genomic sequencing of the *CREBBP* gene in a previous investigation. Two new disease-causing mutations were identified, including a partial deletion of *CREBBP* and a 1-bp deletion in *EP300*, c.7100delC (p.P2366fsX2401). The 1-bp deletion represents the fourth *EP300* mutation reported to date and was identified in a patient with non-classical RSTS. Based on the very similar structure of the *CREBBP* and *EP300* genes and the higher rate of single-nucleotide polymorphisms in *EP300* (2.23 per individual) as compared to *CREBBP* (0.71 per individual) ($P > 0.001$, Wilcoxon test), it may be assumed that *EP300* gene mutations should be as frequent as *CREBBP* gene mutations. Based on the location of the *EP300* gene mutations identified so far (outside the histone acetyl transferase domain) and the observed (although not very striking) phenotypical differences with the *EP300* mutations, we propose that most *EP300* mutations could be associated with other phenotypes, not classical RSTS.

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Introduction

Rubinstein–Taybi syndrome (RSTS) is a well-known autosomal-dominant disorder of typical face, short stature, skeletal abnormalities, and mental retardation, and was shown to be caused in most cases by mutations in *CREBBP*, the gene for CREB binding protein.[1–3] Approximately 45–56% of patients with RSTS demonstrated *CREBBP* mutations,[2,3] and recently, three individuals with *EP300* gene mutations were reported in a series of 92 patients.[4] The *EP300* gene on chromosome 22q13 encodes a protein, p300, that is highly similar to CREBBP. Both CREBBP and p300 are transcriptional coactivators with a plant homeodomain-type zinc finger and a histone acetyl transferase (HAT) domain facilitating chromatin opening.[5,6] They are involved in the regulation of the expression of numerous genes that are important in embryonic development, cell growth, cellular differentiation, and tumor suppression.[7–10] Despite many similarities, CREBBP and p300 are involved in slightly different signal-transduction pathways.[7,9–12] The first report of EP300 mutations in RSTS[4] required a confirmational study (performed here) and raised new questions, for example, regarding the frequency of

*Correspondence: Priv-Doz Dr O Bartsch, Institut für Humangenetik, Universitätsklinikum, Langenbeckstr. 1, D-55101 Mainz, Germany.
Tel: +49 6131 175791; Fax: +49 6131 175690;
E-mail: bartsch@humgen.klinik.uni-mainz.de
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EP300 mutations in RSTS, the causes of the paucity of *EP300 vs CREBBP* mutations, the phenotypic spectrum in humans with EP300 mutations, and possible further genetic heterogeneity in RSTS.

Using genomic sequencing and real-time quantitative PCR (qPCR) we studied 13 patients (out of a series of 38 patients previously reported after fluorescence *in situ* hybridization (FISH) and genomic sequencing of CREBBP),[3] for the presence of *EP300* mutations and *CREBBP* deletions that had escaped detection by FISH. We identified two novel mutations, a 1-bp deletion (c.7100delC) in *EP300* and a *CREBBP* deletion including exons 28 and 31. The *EP300* gene mutation predicts a very mildly truncated protein and does not alter the HAT domain, which elsewhere had been shown to be critical in causing RSTS.[5,6] Based on the location of the *EP300* gene mutations[4] (also this study), the relatively large number of single-nucleotide polymorphisms (SNPs; 2.33 per patient), and the observed (although not very striking) phenotypical differences with the *EP300* mutations detected so far[4] (also this study), we suggest that mutations in *EP300* could be underdiagnosed because they may result in phenotypes different from RSTS. Finally, we identified in *EP300* a homozygous sequence variation (c.2053+8T) that was present in all individuals studied and therefore most likely represents the wild-type sequence.

Materials and methods

Out of a series of 38 previously reported patients, we studied here 13 of 19 patients in whom no *CREBBP* mutation had been identified.[3] Of these, 10 individuals had RSTS and three had a diagnosis of incomplete RSTS (see[3] for definition). From the other six patients without detectable *CREBBP* mutation, there was not enough DNA at disposal.

We used genomic sequencing and real-time qPCR[13,14] for the detection of molecular *EP300* mutations and partial deletions of *CREBBP* and *EP300*, respectively. The 31 coding exons of *EP300* were analyzed in 37 fragments (Table 1) using a previously described protocol[3,15] and a CEQ™ 8000 Genetic Analyzer (Beckman Coulter, Fullerton, CA, USA). All sequence variations were confirmed in a second PCR and sequencing run. The *EP300* gene mutation identified here was proven to be *de novo* by sequencing of DNA from the parents. Paternity testing was not performed due to legal restrictions. The Entrez SNP Database was consulted for interpretation of SNPs. The qPCR was performed using a total of 18 different amplicons (*CREBBP*: intron 1, exon 2, introns 2–3, exon 3, introns 4–5, exon 6, exon 14, exon 16, exon 28, and exon 31; *EP300*: exons 1, 2, 4, intron 9, and exons 14, 18, 25, and 31) and a previously described protocol.[13]

Note that *CREBBP* and *EP300* exon numbers are in line with the literature,[1–6] but differ by −1 from actual exon numbers.

Results

We identified two mutations. In patient 12 described below, genomic sequencing of *EP300* revealed a 1-bp deletion, c.7100delC (p.P2366fsX2401; Figure 1). In patient 21, a 21-year-old woman with RSTS (A-1928–22 in our previous study),[3] qPCR indicated a deletion of *CREBBP* at exons 28 and 31. Thus, the *CREBBP* mutation detection rate increased to 52.6% (20 in 38) in our whole series of patients, or to 60% (18 in 30) in the subset of patients with unequivocal RSTS only.

We also detected five different SNPs in the *EP300* gene (Table 2), all previously reported, including non-synonymous SNPs in exons 3 (c.865A→G, p.M289V, rs2230111) and 15 (c.2989A→G, p.I997V, rs20551), a synonymous SNP in exon 17 (c.3183A→T, rs4822012), and two intronic variants (introns 27–28, c.4452+20C→T, rs6002271; introns 28–29, c.4618–18C→T, rs2076578). Moreover, all patients in this study and two normal controls demonstrated the same homozygous sequence variation at introns 10–11 (c.2053+8G→T, rs6002267) (Figure 1).

Patient 12 (B-3444–13 in our previous study)[3] showing the *EP300* gene mutation was first diagnosed with 'mild variant but unquestionably' RSTS by the late Professor Frank Majewski, Universitätsklinikum Düsseldorf, Germany, who assessed in 2001 that '… of all patients with RSTS whom I have seen (indeed many), she is by far the best as regards intelligence. All others were mentally retarded, but this is certainly not true for this patient. Therefore one would … rather expect an atypical mutation.' *[… Von allen Patienten mit RTS, die ich bisher gesehen habe (wirklich zahlreich!), ist sie mit Abstand die beste, was die Intelligenz betrifft. Alle anderen waren geistig behindert, was bei dieser Patientin sicher nicht zutrifft. Insofern ist … eher mit einer atypischen Mutation zu rechnen.]* At the age of 14 years and 3 months she presented with microcephaly (occipitofrontal circumference 49.5 cm, −3.2 SD) and a very beaked nose, columella below alae nasi, narrow high-arched palate, a very pronounced overbite with the maxillary incisors markedly protruding over the lower lip, and retrognathia. She had mild myopia (−1 D) and broad thumbs and big toes. Height (152.8 cm, −1.4 SD) was normal and intelligence was borderline normal. At age 18 years, the pronounced overbite was corrected by maxillary surgery. She attended an integrated school for the learning disabled, but after 10 classes failed in the final examination. Vocational training was viewless and she now works in a workshop for the mentally handicapped (estimated IQ ~75). The family provided full-length portraits to one of us (OB) but denied permission to publication. The photos

Table 1 Primers and PCR conditions for *EP300* sequencing

EP300 exon (size)	*Position*[a]	*Primer name (fragment size)*	*Primer sequence 5′ → 3′*	*Annealing temperature*
	190, +151	Ex01-F (435 bp)	TTTCTATCGAGTCCGCATCC	53°C
Exon 1 (94 bp)		Ex01-R	ACGTCTTCGACCAGCTCATT	
Exon 2 (635 bp)	−108	Ex02-F1 (536 bp)	GGAGTGAGGTTGGGAAATGA	58.9°C
	428[b]	Ex02-R1	CAGCCAACATTCCAGGATTC	
	320[b]	Ex02-F2 (378 bp)	GGGCACTAGTGGACCAAATC	57.3°C
	58	Ex02-R2	GTAAGGCAAACCCTCCATCC	
Exon 3 (177 bp)	−197, +106	Ex03-F (480 bp)	GCCACCATGTCCAGATTTTT	55.5°C
		Ex03-R	AGTGGGTACAAATCCCAGCA	
Exon 4 (262 bp)	−141, +50	Ex04-F (453 bp)	TGCATTCCCTGTGTCAAAAA	52.3°C
		Ex04-R	TCCCTGGCTGTAAAAATTGC	
Exon 5 (114 bp)	−200, +98	Ex05-F (412 bp)	TTTGTGCAAATTGCTTACCC	55.5°C
		Ex05-R	ACACCACAGGTCCCTCACAT	
Exon 6 (246 bp)	−187, +49	Ex06-F (482 bp)	CATTTACTGACACAACCAATACCA	55.5°C
		Ex06-R	TGGTCCCCTTTACCAATCAG	
Exon 7 (94 bp)	−263, +103	Ex07-F (460 bp)	GCCTGGTCACATTTGCTTTT	52.3°C
		Ex07-R	GACATCCTCAAACCGAGGAA	
Exon 8 (138 bp)	−145, +157	Ex08-F (440 bp)	CACACTTCTCCCTGCCTAGC	55.5°C
		Ex08-R	CAGCAGCAAGAAATCCACAA	
Exon 9 (118 bp)	−298, +50	Ex09-F (466 bp)	TGACAAGGCCTGTTTTCCTC	55.5°C
		Ex09-R	CCACAACAGGTTCAATCTTGG	
Exon 10 (175 bp)	−126, +130	Ex10-F (431 bp)	TTGGCACCAGTTCTTAATGC	52°C
		Ex10-R	TTTGCAAAGGCAAATGGTTA	
Exon 11 (78 bp)	−200, +173	Ex11-F (451 bp)	TCCACTTGGAGGCATTTTTC	55.5°C
		Ex11-R	AATGTCACCTGCCCTGTGAT	
Exon 12 (110 bp)	−189, +200	Ex12-F (499 bp)	GAATGGTGTGAACCCAGGTG	55.5°C
		Ex12-R	CACTGACACTCCAGGGACAA	
Exon 13 (138 bp)	−184, +95	Ex13-F (417 bp)	TGGCACAAGAGTCAGTTGTTCT	53°C
		Ex13-R	ACCCACTATTTGCTGCCACT	
Exon 14 (791 bp)	−180, +58	Ex14-F (553 bp)	CTTGATGGTGCTGTCCAAAG	57°C
		Ex14-R	GAATGGAAATGGCCCAGAAG	
Exon 15 (180 bp)	−154, +132	Ex15-F (466 bp)	GTGAAATGGGCAGAGCAAAT	52.3°C
		Ex15-R	GAACACCCTTTCTCATGCAA	
Exon 16 (145 bp)	−214, +127	Ex16-F (486 bp)	CAGCCGGAGGATATTTCAGA	55.5°C
		Ex16-R	CAGGGCTTCCAGGTTAATGA	
Exon 17 (119 bp)	−146, +72	Ex17-F (337 bp)	GAGCCTGTAGTGATATTTCC	55°C
		Ex17-R	GAGTGGCTATACTGTTTGG	
Exon 18 (240 bp)	−138, +105	Ex18-F (483 bp)	TCAGTCACCTCTTGGGGAATA	53.1°C
		Ex18-R	AGCCATGTCAAAGCCAGAAG	
Exon 19 (89 bp)	−190, +205	Ex19-F (484 bp)	AGCCTACCTCAGCGTTTTGA	55.5°C
		Ex19-R	CCCAGGTAACTGATGCAAGG	
Exon 20 (147 bp)	−94, +177	Ex20-F (418 bp)	CGGAACAGTTCACCCCAGTA	53°C
		Ex20-R	GCCCATTGCTGACATATTCC	
Exon 21 (81 bp)	−38, +301	Ex21-F (420 bp)	GGGTGAAGTTTGTTCCTTTGG	55.5°C
		Ex21-R	TTCCTGGTGCTGAAAATTCC	
Exon 22 (57 bp)	−87, +348	Ex22-F (492 bp)	TTGGAATTGGCTCTGCTCTT	55.5°C
		Ex22-R	ACCTCGCCTGGCCATAAATA	
Exon 23 (78 bp)	−24, +312	Ex23-F (414 bp)	ATGCCCTTCATGTTTCTTCA	55.5°C
		Ex23-R	TGCATTCTACAAATCCGTTCC	
Exon 24 (68 bp)	−216, +215	Ex24-F (499 bp)	TTAGCATGTTCCCTGCACTC	51.5°C
		Ex24-R	CTGCCATCTCTCCACTGTCC	
Exon 25 (151 bp)	−189, +147	Ex25-F (487 bp)	CTCGTGGATCCAAAATTGCT	55.5°C
		Ex25-R	TATTTTGTTTGGGGGTTTGC	
Exon 26 (114 bp)	−199, +151	Ex26-F (464 bp)	AGAAGGAAACCACAGGCTCA	56.2°C
		Ex26-R	AAAGGGGCTCCAACAAAGTT	
Exon 27 (166 bp)	−168, +164	Ex27-F (498 bp)	GCCTAATTTTGGCCTCACAA	56.2°C
		Ex27-R	GAGCCAAAATCGTGTCACTG	
Exon 28 (165 bp)	−116, +200	Ex28-F (481 bp)	TGCCAGCTTTCAAGACATTTT	53°C
		Ex28-R	AAAAGGGGCTCTGATGCTTT	
Exon 29 (162 bp)	−285, +48	Ex29-F (495 bp)	TAGCCCCAATCTGGGATACA	53°C
		Ex29-R	GCCAGAAATCTTGCCGTTT	
Exon 30 (282 bp)	−104, +76	Ex30-F (462 bp)	CCAGGAGGCAGAGGTTGTAG	59°C
		Ex30-R	AGCATCCCACAGGCCTCTAT	
Exon 31 (2184 bp)	−95	Ex31-F1 (417 bp)	ACGAAAGGGGCTTTTCTAGC	54.7°C
	322[b]	Ex31-R1	GCATTTGTTCTCCTGGCAGT	
	247[b]	Ex31-F2 (500 bp)	GTTGCAAACGGAAAACCAAT	54.7°C
	747[b]	Ex31-R2	CGCTGCTCTCTGAATCTGC	

Table 1 (*Continued*)

EP300 exon (size)	*Position*[a]	*Primer name (fragment size)*	*Primer sequence 5′ → 3′*	*Annealing temperature*
	658[b]	Ex31-F3 (471 bp)	CCCTCAGGTTCATCTTGCAT	55.5°C
	1129[b]	Ex31-R3	GAGAGCTGGGAGACCTGAGA	
	1032[b]	Ex31-F4 (490 bp)	GGATTGGGCCAGGTAGGTAT	54.7°C
	1522[b]	Ex31-R4	GTTGCTGCTGCTGTTGCAT	
	1474[b]	Ex31-F5 (487 bp)	TCCGAGACATCTTGAGACGA	54.7°C
	1961[b]	Ex31-R5	GAAACGTGGTGTGGAGAAGG	
	1869[b]	Ex31-F6 (435 bp)	CTTCTCCACGGCCACAGT	57.5°C
	+120	Ex31-R6	ACGGCATACTGCACAGTTCTT	

[a]Position of left and right primers relative to first and last nucleotide of exon, respectively.
[b]Position within exon, given relative to first nucleotide of exon 31.

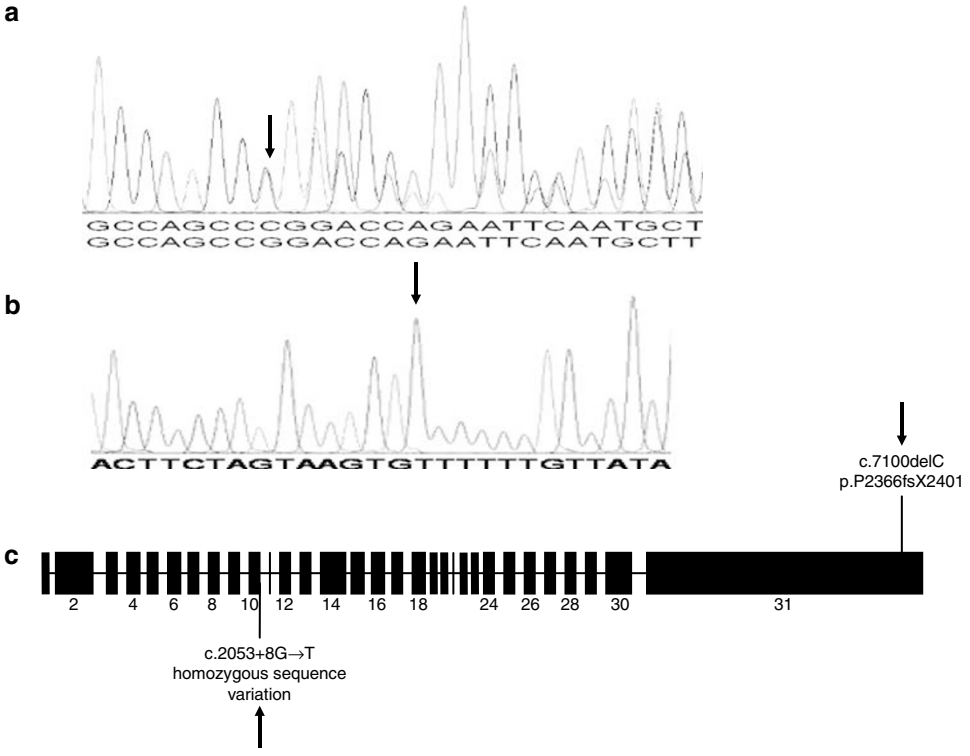

Figure 1 *EP300* gene, (**a**) the 1-bp deletion, c.7100delC, identified in patient 12 with mild variant RSTS and borderline intelligence. (**b**) The thymine residue at position 8 of the exon 10 splice donor (c.2053 + 8T), which was previously reported as a SNP (c.2053 + 8G→T, rs6002267), but identified in homozygous state in all study patients and two healthy controls, suggesting that thymine is the wild-type sequence. (**c**) Position of the c.7100delC (p.P2366fsX2401) mutation and of the c.2053 + 8T 'SNP' within the *EP300* gene.

showed no classical RSTS, as also evident from the pronounced overbite and orthodontic surgery performed here.

Discussion

The c.7100delC deletion in exon 31 represents the fourth disease-associated mutation in *EP300* reported to date and the first confirmation of the results of Roelfsema *et al*[4] It predicts a premature stop codon, p.P2366fsX2401, and a protein truncation towards the 3′-end (normal size, 2414 residues). Roelfsema *et al*[4] described two *EP300* mutations (one nonsense, one frameshift) predicting small truncated proteins without the HAT domain, and a third mutation probably deleting essential parts of the *EP300* promotor region and probably not resulting in any protein at all. The c.7100delC deletion occurred *de novo* and in all likelihood represents the causative mutation in our patient, not a polymorphism. Frameshift and nonsense mutations affecting the HAT domain represent the most frequent mutation types observed in RSTS with the *CREBBP*[1–3,5,6] and *EP300*[4] genes. However, the location of the present frameshift

Table 2 Twenty-nine single-nucleotide polymorphisms of the *EP300* gene, including three homozygous SNPs, detected in 13 patients

Patient[a]	*Internal code*[a]	*Location*	*Mutation*		*dbSNP rs#*[b]
24	B-3510-25	Exon 3	c.865A→G	Non synonymous, p.M289V	2230111
8	A-3435-09	Exon 15	c.2989A→G	Non synonymous, p.I997V	20551
12	B-3444-13	Exon 15	c.2989A→G[c]	Non synonymous, p.I997V	20551
13	B-3446-14	Exon 15	c.2989A→G	Non synonymous, p.I997V	20551
18	A-3478-19	Exon 15	c.2989A→G	Non synonymous, p.I997V	20551
33	A-3584-35	Exon 15	c.2989A→G	Non synonymous, p.I997V	20551
10	A-3437-11	Exon 17	c.3183A→T	Synonymous	4822012
13	B-3446-14	Exon 17	c.3183A→T	Synonymous	4822012
15	A-3463-16	Exon 17	c.3183A→T	Synonymous	4822012
20	A-1878-21	Exon 17	c.3183A→T	Synonymous	4822012
24	B-3510-25	Exon 17	c.3183A→T	Synonymous	4822012
33	A-3584-35	Exon 17	c.3183A→T	Synonymous	4822012
44	A-3639-47	Exon 17	c.3183A→T	Synonymous	4822012
8	A-3435-09	Exon 27-28	c.4452+20C→T	Intron	6002271
12	B-3444-13	Exon 27-28	c.4452+20C→T	Intron	6002271
13	B-3446-14	Exon 27-28	c.4452+20C→T	Intron	6002271
18	A-3478-19	Exon 27-28	c.4452+20C→T	Intron	6002271
33	A-3584-35	Exon 27-28	c.4452+20C→T	Intron	6002271
7	A-3433-08	Exon 28-29	c.4618-18C→T[c]	Intron	2076578
10	A-3437-11	Exon 28-29	c.4618-18C→T	Intron	2076578
15	A-3463-16	Exon 28-29	c.4618-18C→T	Intron	2076578
20	A-1878-21	Exon 28-29	c.4618-18C→T	Intron	2076578
21	A-1928-22	Exon 28-29	c.4618-18C→T	Intron	2076578
24	B-3510-25	Exon 28-29	c.4618-18C→T	Intron	2076578
27	A-3508-28	Exon 28-29	c.4618-18C→T[c]	Intron	2076578
44	A-3639-47	Exon 28-29	c.4618-18C→T	Intron	2076578

[a]Patient and code numbers same as in previous publication; Bartsch *et al*.[3] letters A or B in internal code indicate phenotype: A = RSTS, B = incomplete RSTS.
[b]Polymorphism previously reported in the Entrez Single-Nucleotide Polymorphism Database (dbSNP).
[c]Homozygous SNP.

mutation very close to the 3′-end of *EP300* is interesting;[3,5,6,11,16] it leads to a putative very mild truncation that does not alter the HAT domain, and could possibly explain the non-classical RSTS (unusual face, relatively good intelligence) in our patient. An overbite with the maxillary incisors markedly protruding over the lower lip and requiring maxillary surgery is an unusual feature in RSTS.[17]

We identified only one *EP300* mutation in this series of 38 patients, or ~2.6%, a frequency corresponding well with the previous report of ~3.2% *EP300* mutations.[4] Considering the similar genetic properties and functions of *CREBBP* (31 exons, 2442 residues) and *EP300* (31 exons, 2414 residues), one might expect similar rates of mutations and similar sites of pathogenic mutations. Hence, the 20-fold difference (52.6 *vs* 2.6%) between the rates of detectable mutations in *CREBBP* and *EP300* in our patients strongly suggests that *EP300* plays only a minor role in the etiology of RSTS. The relatively low total number of mutations identified in *CREBBP* and *EP300* (60%, considering the most stringent diagnostic group A) could be in support of further genetic heterogeneity in RSTS.

Furthermore we detected in the *EP300* gene five SNPs (Table 2; two non-synonymous SNPs, a synonymous SNP and two intronic variants). Addressing the lower rate of pathogenic mutations in *EP300* as compared to *CREBBP*, Roelfsema *et al*[4] discussed the possibility of different chances of mutations occurring in these two genes, despite their very similar genetic composition (31 coding exons, ~2400 residues). Alternatively, mutation rates could be similar and the vast majority of individuals with *EP300* mutations could demonstrate phenotypes other than RSTS. In our previous study,[3] we identified 15 different SNPs in *CREBBP* in 38 patients (0.39 per individual) and here we detected five different *EP300* SNPs in 13 patients (0.38 per individual), a finding supporting approximately equal mutation rates in the two genes. However, the 13 patients in this study demonstrated a total of 29 SNPs in *EP300* (Table 2), or 2.23 per individual, as compared to 27 *CREBBP* polymorphisms in 38 patients, or 0.71 per individual, identified in our previous study.[3] Hence, *EP300* polymorphisms were present at higher frequencies (Wilcoxon test, $p < 0.001$). The higher frequencies of the *EP300* SNPs could possibly reflect differences in genetic drift of the different polymorphisms. Taken together, our data support that *EP300* mutations should be roughly about as frequent as *CREBBP* mutations, but some could lead to other phenotypes, not classical RSTS.

Similarly, the very different frequencies of *CREBBP* and *EP300* mutations with the RSTS phenotype and the observed (although not very striking) phenotypical differences with the *EP300* mutations[4] (also this study) suggest that mutations in *EP300* could be underdiagnosed due to phenotypes different from classical RSTS. A possible explanation could be the different functions of CREBBP and EP300 during embryogenesis.[7,9,18]

Finally, a homozygous sequence variation (*EP300* c.2053 + 8T) was present in all individuals studied and hence most likely represents the wild-type sequence. We therefore suggest that the guanine at position 8 of the exon 10 splice donor in the current version of the human genome reference sequence represents a polymorphism.

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Electronic-database information

URLs and accession numbers for data in this article are as follows: Online Mendelian Inheritance in Man (OMIM, http://www.ncbi.nlm.nih.gov/Omim) for the Rubinstein–Taybi syndrome (MIM 180849). Ensembl (http://www.ensembl.org/Homo_sapiens/index.html) for *CREBBP* and *EP300* sequences. Single-Nucleotide Polymorphism Database (dbSNP, http://www.ncbi.nlm.nih.gov/SNP/) for SNPs in *EP300* and *CREBBP*. Primer 3 Output software (http://www.genome.wi.mit.edu//cgi-bin/primer/primer3_www.cgi).

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