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Phenotype and Genotype in 52 Patients with Rubinstein–Taybi Syndrome Caused by *EP300* Mutations

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Rubinstein–Taybi syndrome (RSTS) is a developmental disorder characterized by a typical face and distal limbs abnormalities, intellectual disability, and a vast number of other features. Two genes are known to cause RSTS, *CREBBP* in 60% and *EP300* in 8–10% of clinically diagnosed cases. Both paralogs act in chromatin remodeling and encode for transcriptional co-activators interacting with >400 proteins. Up to now 26 individuals with an *EP300* mutation have been published. Here, we describe the phenotype and genotype of 42 unpublished RSTS patients carrying *EP300* mutations and intragenic deletions and offer an update on another 10 patients. We compare the data to 308 individuals with *CREBBP* mutations. We demonstrate that *EP300* mutations cause a phenotype that typically resembles the classical RSTS phenotype due to *CREBBP* mutations to a great extent, although most facial signs are less marked with the exception of a low-hanging columella. The limb anomalies are more similar to those in *CREBBP* mutated individuals except for angulation of thumbs and halluces which is very uncommon in *EP300* mutated individuals. The intellectual disability is variable but typically less marked whereas the microcephaly is more common. All types of mutations occur but truncating mutations and small rearrangements are most common (86%). Missense mutations in the HAT domain are associated with a classical RSTS phenotype but otherwise no genotype–phenotype correlation is detected. Pre-eclampsia occurs in 12/52 mothers of *EP300* mutated individuals versus in 2/59 mothers of CREBBP mutated individuals, making pregnancy with an *EP300* mutated fetus the strongest known predictor for pre-eclampsia.

Key words: *EP300*; Rubinstein–Taybi syndrome; phenotype; genotype; pre-eclampsia

INTRODUCTION

Rubinstein–Taybi syndrome (RSTS; OMIM 180849) is one of the archetypical intellectual disability—multiple congenital anomaly syndromes [Rubinstein and Taybi, 1963]. Major characteristics are intellectual disability, unusual face, broad thumbs and halluces, increased risk to develop tumors, and typical behavior [Hennekam, 2006]. RSTS was found to be caused first by mutations in or deletions of CREBBP [Petrij et al., 1995]. The phenotype of RSTS individuals with a CREBBP mutation has been reported repeatedly and is well known [Bartsch et al., 2005; Schorry et al., 2008; Rusconi et al., 2015; Spena et al., 2015; Wincent et al., 2015]. Subsequently, mutations in *EP300* were detected in individuals clinically diagnosed as having RSTS [Roelfsema et al., 2005]. *EP300* and *CREBBP* are paralogs and their proteins belong to the family of histone-acetyl-transferases and act as global transcriptional co-activators [Roelfsema and Peters, 2007]. Although similar for over 70%, there are functional differences between the encoded protein, CREBBP and P300, when deciphering their interactions with different transcription factors involved in non-redundant pathways [Roelfsema and Peters, 2007]. No mutations can be found in the remaining ~30% of reliably diagnosed RSTS individuals and the cause of the syndrome in them remains at present unknown.

Up to now 26 individuals with RSTS and an EP300 mutation have been described [Roelfsema et al., 2005; Bartholdi et al., 2007; Zimmermann et al., 2007; Foley et al., 2009; Bartsch et al., 2010; Tsai et al., 2011; Woods et al., 2014; Bounakis et al., 2015; Masuda et al., 2015; Negri et al., 2015, 2016; Solomon et al., 2015; Wincent et al., 2015; Tamhankar et al., 2016]. This limited information on the phenotype hampers informing and counseling families adequately. Here, we report on 52 RSTS individuals with an *EP300* mutation (42 unpublished) and compare data to reported individuals with a *CREBBP* mutation. We evaluate possible genotype–phenotype correlations, and pay particular attention to the frequency of pre-eclampsia in pregnant mothers carrying a child with an *EP300* mutation [Van Uitert et al., 2015].

METHODS

Patients

The RSTS patients described here were gathered through the laboratories in Europe that offer *EP300* testing on a diagnostic basis (Bordeaux, Leiden, Mainz, Milano, Southampton). In most patients, first studies for a *CREBBP* mutation were performed before the *EP300* studies were performed, but in some patients *EP300* mutation analysis was performed as first study, especially in individuals with a higher level of functioning. In addition, three patients were referred to one of us (RCH) because of an unusual experience in RSTS, and the finding of an *EP300* mutation in a child using whole exome sequencing targeted for all genes known to cause intellectual disability.

Informed consent was obtained from the parents or legal representatives of all patients. Subsequently, using a questionnaire clinical and molecular information was obtained from the clinicians. If the parents of patients had allowed this, clinical pictures and in some also radiographs were obtained and evaluated by two of us (DL; RCH). Ten patients have been published but follow-up data and more detailed information have become available and are presented here. These patients are here reported as patient 18 and 19 [Bartsch et al., 2010], patient 25 [Solomon et al., 2015], patient 29 [Negri et al., 2015 (patient #54)], patient 32 and 33 [Wincent et al., 2015], and patients 38, 39, 40, and 48 [Negri et al., 2016 patients #39, #65, #256, #207, respectively)].

The Medical Ethics Committee of the Academic Medical Centre in Amsterdam approved the study.

Molecular Analyses

Molecular testing was performed in each of the diagnostic laboratories following their routine procedures. Mutation screening was performed either by Denaturing High Performance Liquid Chromatography or High Resolution Melting followed by Sanger sequencing (ABI, Life technologies) of all the exons showing an abnormal profile or by directly sequencing all exons. In two patients, CREBBP and EP300 were sequenced together using a dedicated custom gene panel (ThermoFisher). After AmpliSeq library synthesis and emPCR new generation sequencing was performed on a PersonalGenome Machine (Ion Torrent, ThermoFisher). Gene dosage was analyzed using Multiplex by Ligation Polymerase chain reaction Assay or targeted array Comparative Genome Hybridization (CGH) and semi Quantitative Multiplex Fluorescent Polymerase Chain Reaction (QMF-PCR). All missense variants were evaluated using the Alamut interface (Interactive Biosoftware).

Statistical Analyses

The Fisher's exact test was used for all comparisons taking into account the low number of patients in some groups with $P < 0.05$ considered significant.

RESULTS

The phenotypes of the presently studied individuals are presented in Table I and illustrated in Figure 1A summary of the findings is compared to the findings in RSTS patients with a *CREBBP* mutation in Table II. The description is based on the features observed in *CREBBP* mutated patients, facial characteristics, thumbs and halluces morphology, growth retardation and psychomotor developmental delay, and as additional feature pre-eclampsia in mothers. The mutations of the present cohort are provided in Table I for each patient and graphically summarized in Figure 2. Below only findings not mentioned in the Tables are added.

Phenotype

One patient has a cleft lip and palate. Two patients had talon cusps. Posterior helical pits were found in four of the patients. Among ocular anomalies, strabismus (17/44) and myopia (10/42) were the most frequent. Astigmatism and hyperopia were observed in three and two cases, respectively and anterior chamber anomaly in two cases. Additional ocular anomalies, each reported once, were: ptosis, coloboma, cataract, congenital nystagmus, small optic nerves, and lacrimal duct stenosis. One patient had an asymmetry

TABLE I. Phenotype and Genotype of 52 Patients With an *EP300* Mutation

Patient	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13
Sex	F	M	M	F	M	F	M	M	M	F	F	F	M
Prenatal growth retardation	-	+	+	-	-	+	-	+	-	-	-	na	+
Pre-eclampsia	-	-	-	-	-	-	-	-	-	+	-	-	+
Postnatal growth retardation	+	+	+	+	+	+	+	-	+	+	+	-	-
Microcephaly	+	+	+	+	+	-	+	+	-	-	+	+	+
Hypertrichosis	+	+	na	na	na	+	-	+	+	-	+	+	+
Low anterior hairline	+	-	+	+	+	+	+	+	+	-	+	-	-
Arched eyebrows	-	+	+	+	+	+	+	-	-	+	+	+	+
Long eyelashes	+	+	+	-	+	+	+	+	-	+	+	-	na
Narrow forehead	+	-	+	+	+	+	-	+	+	+	na	-	-
Convex nasal ridge	+	-	+	+	+	+	-	+	+	+	+	+	+
Large nose	+	+	+	+	+	+	-	-	+	+	+	-	+
Columella below alae nasi	+	+	+	+	+	+	+	+	+	+	+	+	+
Slanting palpebral fissures*	D	D	D	D	D	D	D	H	H	D	H	H	H
Epicanthal folds	-	+	-	-	+	-	+	-	-	+	-	-	-
Highly arched palate	-	-	+	+	-	+	+	+	+	+	+	-	na
Micrognathia	+	-	-	+	-	+	+	-	-	+	+	-	+
Low-set ears	-	-	-	+	+	-	+	+	-	+	+	-	+
Pits posterior helix	-	+	-	na	na	-	na	na	-	-	-	-	na
Broad thumbs	-	-	+	+	+	+	-	+	-	+	+	-	+
Angulated thumbs	-	-	-	-	-	-	-	-	-	-	-	-	-
Broad halluces	+	+	+	+	+	+	+	-	+	+	+	-	+
Intellectual disability**	-	+ ns	+ ns	+ ns	+ ns	Mo	+ ns	Mi	Mo	+ ns	S	-	na
Epilepsy	-	-	-	-	-	-	-	-	-	-	+	+	na
Autism/autistiform behavior	+	-	-	-	-	-	na	-	-	+	+	-	na
Other behavioural problems	Personality disorder	Aggression, disturbed sleep	-	Hyperactivity	-	na	-	Hyperactivity, anxieties	Stereotypy, temper tantrums, disturbed sleep	Self injuring	Self injuring	-	na
Cardiovascular anomalies***	-	-	MVD	-	MVD / AVD	VSD	-	-	-	-	TF	A	na
Urinary tract anomalies****	-	DK	-	-	-	R	-	-	-	R	AK	-	-
Scoliosis	-	-	-	-	-	-	-	+	-	-	+	na	na
Obesity	-	-	-	+	-	+	-	+	+	-	+	+	-
Keloids	-	-	-	-	-	+	-	-	-	-	-	+	-
Pilomatricoma	+	-	-	na	na	+	na	na	-	-	na	+	-
Other		gut paresis			Small optic nerves, PE*****, syringomelia, broad distal phalangae fingers, syndactyly toe2+3								
***EP300* mutation**	c.1553_1554dup, p.Gly519fs	c.3071_3074del, p.Lys1024fs	c.3234del p.Val1079fs	c.4238T>A p.Val1413Asp	c.3857A>G p.Asn1286Ser	c.4946G>A, p.Trp1649*	c.2554C>T p.Gln852*	c.6347del p.Pro2116fs	c.5506C>T p.Gln1836*	c.4078_4086del, p.Leu1360_Ala1362del	c.4301A>G p.His1434Arg	c.2113C>T p.Arg705*	c.4026-9A>G p.?

TABLE I. (*Continued*)

# 14	# 15	# 16	# 17	# 18	# 19	# 20	# 21	# 22	# 23	# 24	# 25	# 26	# 27
M	M	F	F	M	F	M	M	M	M	M	F	M	M
-	+	+	+	-	-	-	na	+	-	+	+	+	-
-	+	-	-	-	-	-	+	-	-	-	+	-	-
+	+	+	+	-	+	na	-	-	-	-	+	-	+
+	+	+	+	+	+	+	-	+	+	+	+	+	+
+	-	-	+	-	na	-	-	+	-	-	+	+	+
-	-	+	-	-	na	-	+	+	+	+	-	+	+
-	+	+	+	-	+	-	-	+	+	+	-	+	+
+	+	+	+	na	+	+	+	+	+	+	-	+	+
+	-	-	-	na	na	-	-	+	-	+	-	-	-
+	+	+	-	-	+	+	+	+	-	-	-	-	-
+	+	-	-	-	+	+	-	+	-	-	+	-	+
+	+	+	+	-	+	+	+	-	+	+	+	+	+
D	D	D	H	H	H	H	D	D	D	H	H	H	H
-	-	-	-	-	-	-	-	+	-	-	-	-	-
+	+	+	+	na	+	-	+	+	+	-	-	-	-
+	+	-	+	+	+	-	-	-	-	+	-	-	+
+	-	+	-	-	-	-	-	+	-	-	-	-	-
-	na	na	-	+	-	-	-	-	na	-	-	-	na
+	-	+	+	+	+	-	+	+	+	-	+	-	+
-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	-	-	+	+	+	-	+
Mo	Mi	Mi	Mo	+ ns	+ ns	+ ns	+ ns	Mi	Mi	Mo	+ ns	Mi	+ ns
-	-	-	-	-	-	na	-	-	-	+	-	-	-
-	-	-	+	-	-	-	+	+	+	-	-	-	-
-	Hyperactivity	Aggression	-	Hyperactivity, self injuring	na	na	+ ns	+ ns	-	+ ns	-	Obsessive	Hyperactivity
-	VSD	-	PS / MS	-	VSD	-	-	PSVC	-	-	-	-	-
R	-	BK/BP	BP	-	na	-	-	-	-	-	AK	-	-
-	+	+	+	-	+	na	-	-	-	-	-	-	-
-	-	+	-	-	-	-	+	-	-	+	+	+	-
-	na	na	-	-	-	-	-	-	-	-	-	+	-
-	na	-	-	-	-	-	-	+	-	-	-	-	-
			spina bifida occulta		na		obstructive sleep apnea		talon cusps	syndact toe2+3	sacral lipoid myelomeningocele	talon cusps	
interstitial deletion Hg19 g.41556074_41563532	c.4578_4579insTT p.Arg1527fs	c.1167dup p.Val390fs	c.256C>T p.Arg86*	c.638del p.Gly213fs(HGMD CD100124)	c.7100delC p.Pro2367fs	c.1187_1189delinsATT p.Ser396_Arg397delinsTyr*	c.2251_2257del p.Tyr751fs	c.4954_4957dup p.Met1652fs	c.4390C>T p.Gln1464*	c.1975_1976del p.Pro659fs	c.4933C>T p.Arg1645*	c.1876C>T p.Arg626*	c.718dupC p.Gln240fs

TABLE I. (*Continued*)

M	M	M	F	M	M	M	M	M	F	M	M	F	M	M
+	+	na	-	-	-	-	-	+	na	+	-	-	-	-
+	-	+	-	+	-	-	-	-	-	-	-	-	-	+
-	+	-	-	+	-	-	+	-	+	+	+	+	-	na
-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
-	-	na	+	-	na	-	+	+	na	-	+	-	+	-
+	-	-	+	-	+	+	+	+	+	-	+	-	+	-
+	-	-	+	+	+	+	+	+	-	-	+	-	+	+
+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
-	-	-	-	-	-	+	-	+	+	-	-	-	-	+
-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D	H	D	H	D	D	H	D	U	U	D	D	H	D	D
-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
+	na	+	+	+	na	na	+	+	-	+	+	+	-	-
+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
na	na	-	-	-	na	-	-	-	na	-	+	-	+	-
-	-	-	+	+	+	+	+	+	+	-	+	-	+	-
-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
-	-	+	-	+	+	+	+	+	+	+	+	+	+	+
Mi	+ ns	Mi	+ ns	Mi	+ ns	+ ns	Mi	Mi	+ ns	Mi	Mi	+ ns	Mo	Mi
-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	+	na	-	-	-	-	-	-	-	-	+
Hyperactivity	Anxieties	+ ns	-	Self injuring, temper tantrums	na	-	-	-	+ ns	Anxieties	Hyperactivities	Anxieties, temper tantrums	Hyperactivities	-
-	na	-	TF	VSD	na	-	-	na	-	-	-	-	-	-
-	-	-	-	-	-	-	R	na	-	-	-	-	-	-
+	-	-	-	-	-	-	+	+	-	-	+	-	-	-
-	-	+	-	-	-	+	-	-	+	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	multiple nevi, overdeveloped nipples		persistent hypokalaemia		PE			PE; GE reflux	cleft lip/palate		PE, partial syndactactyly fingers, , multiple nevi			
c.3728+5G>C	c.669dupT	Interstitial deletion	c.3438_3439del	c.4783_4784delTT	c.5824A>T	c.2437C>T	c.1371_1374del	c.4354_4359del	c.4912dup	c.4640dup	c.3829A>T	c.4764_4765del	c.4585C>T	c.2966delC
p.?	p.Gln223fs	c.1529-?1622+?	p.Glu1147fs	p.Phe1595fs	p.Met1942Leu	p.Gln813*	p.Ser457fs	p.Pro1452_Pro1453del	p.His1638fs	p.Asn1547fs	p.Lys1277*	p.Met1588fs	p.Arg1529*	p.Pro989fs

TABLE I. (*Continued*)

# 43	# 44	# 45	# 46	# 47	# 48	# 49	# 50	# 51	# 52	Frequency	observed:collected	**Patient**
F	M	F	F	F	M	F	M	M	M	sex ratio: 34/18		Sex
+	+	-	+	-	+	-	-	-	-	44%	20:48	Prenatal growth retardation
+	-	-	-	-	-	-	-	-	+	21%	11:52	Pre-eclampsia
+	+	+	+	+	+	-	-	-	+	63%	32:50	Postnatal growth retardation
-	+	+	+	+	+	+	+	+	+	86%	45:52	Microcephaly
-	+	-	-	+	-	-	+	+	-	51%	23:45	Hypertrichosis
-	+	+	+	+	-	-	+	+	-	60%	30:50	Low anterior hairline
+	-	+	-	+	-	-	+	+	-	65%	34:52	Arched eyebrows
+	+	+	-	+	+	+	+	+	+	90%	44:49	Long eyelashes
-	+	+	+	+	+	+	+	+	-	47%	23:49	Narrow forehead
-	+	+	-	-	+	-	-	-	+	43%	23:52	Convex nasal ridge
-	-	+	-	-	+	-	+	+	+	44%	23:52	Large nose
+	+	+	-	+	+	-	+	+	+	92%	48:52	Columella below alae nasi
H	H	D	D	D	D	U	D	D	U	56% 36% 8%	28:52	Slanting palpebral fissures*
-	-	-	-	-	-	-	-	+	-	14%	7:52	Epicanthal folds
-	+	+	+	+	+	-	na	-	na	67%	30:45	Highly arched palate
+	-	+	-	-	-	+	+	-	+	42%	22:52	Micrognathia
-	-	+	-	-	-	-	-	+	-	27%	14:52	Low-set ears
-	-	na	-	-	na	-	-	-	-	11%	4:37	Pits posterior helix
+	+	+	-	+	+	+	-	+	+	69%	36:52	Broad thumbs
-	-	-	-	-	-	-	-	-	-	2%	1:51	Angulated thumbs
+	-	+	-	+	+	+	+	+	+	81%	42:52	Broad halluces
-	S	Mo	Mo	Mi	Mo	Mi	+ ns	+ ns	Mi	94%	48:51	Intellectual disability**
-	-	-	+	-	-	-	-	-	-	10%	5:50	Epilepsy
-	-	-	-	+	+	+	-	-	-	25%	12:49	Autism/autistiform behavior
-	-	Disturbed social interactions	Hyperactivity, disturbed social interactions	Aggression	Obsessive	Anxieties	Stereotypy	na	-	65%	30:46	Other behavioural problems
-	-	na	-	-	na	-	-	+	-	26%	12:47	Cardiovascular anomalies***
-	-	na	-	-	na	RI	na	-	R	24%	11:45	Urinary tract anomalies****
-	-	+	-	-	+	-	-	-	-	25%	12:49	Scoliosis
-	+	+	+	+	-	+	+	-	-	39%	20:52	Obesity
-	+	-	+	-	na	-	-	-	-	10%	5:49	Keloids
-	-	-	+	-	na	-	-	-	-	11%	5:45	Pilomatricoma
long fingers, recurrent marked anemia, sarcoidose-like changes lungs	thrombocytopenia	broad distal finger phalanges; breast cancer at age 55yr	broad distal finger phalanges; premature telarche	asymmetric mammae	reflux	PE		Large cisterna magna, bilateral post axial hexadactyly hands				other
Interstitial deletion	c.4371_4376del	c.4066C>T	c.1876C>T	c.1833T>G	c.1879-12A>G	c.3507_3511delinsAG	c.104_107del	c.4774A>T	c.2053+1G>C			
c.3875-?_4779+?	p.Ile1457_Lys1459delinsMet	p.Arg1356*	p.Arg626*	p.Tyr611*	p.?	p.Phe1170_Ser1171delinsAla	p.Ser35fs	p.Lys1592*	p.?			

*U, upward; D, downward; H, horizontal.
**Mi, mild; Mo, moderate; S, severe; ns, not specified.
***VSD, ventricular septal defect; MVD, mitral valve dysplasia; AVD, aortic valve dysplasia; TF, tetralogy of Fallot; A, congenital aortic aneurysm; PS, pulmonic stenosis; MS, mitral stenosis.
****DK, duplication of a kidney; R, versicoureteral reflux; AK, agenesis of a kidney; BK, bifid kidney; BP, bifid pyelum; RI, recurrent infection PE, pectus excavatus; na, information not available; +, present; −, absent; ns, not specified.

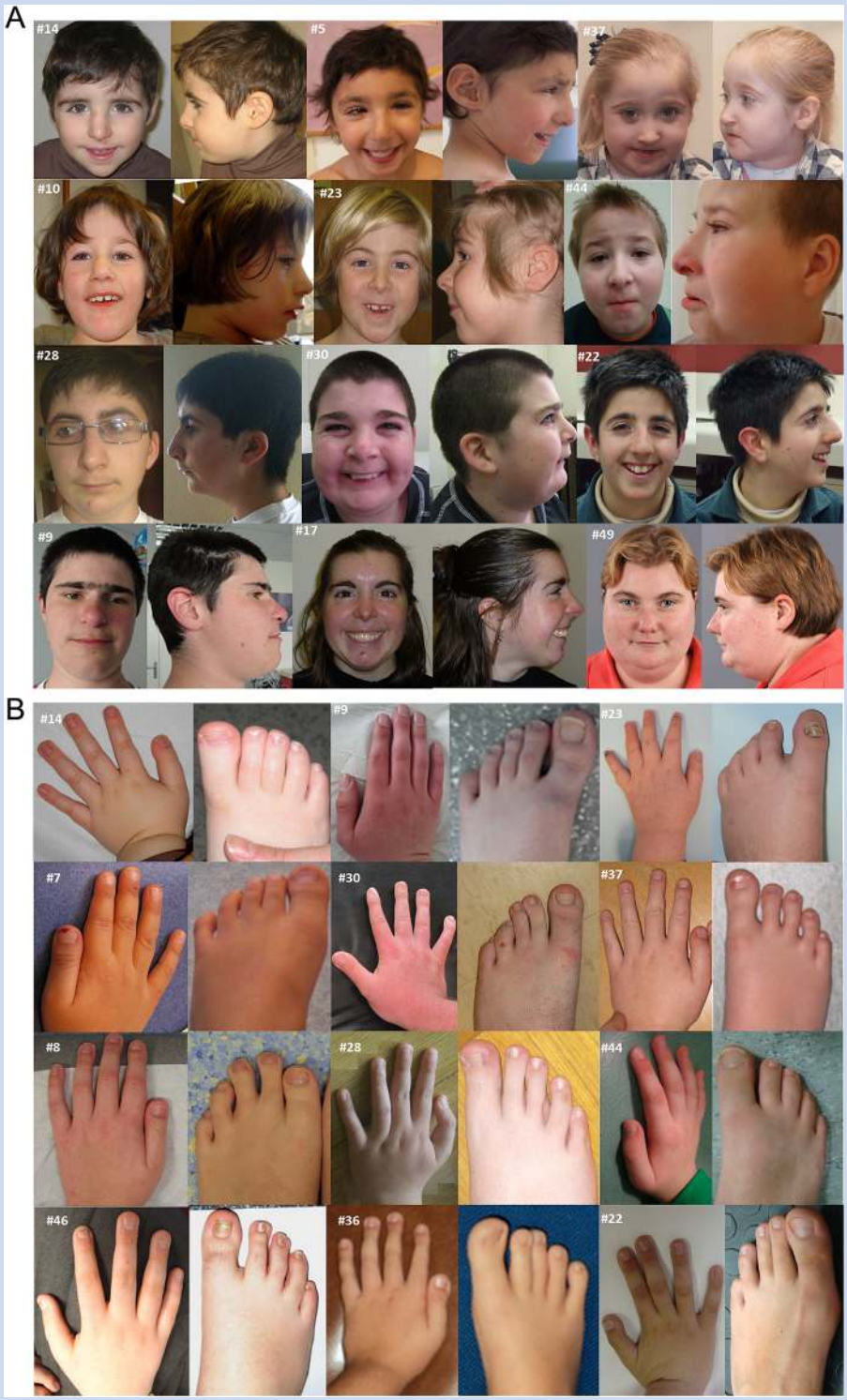

FIG. 1. A: Patients with *EP300* mutations exhibiting typical (#14, #5, #10, #22, #30), somewhat typical (#17,#28, #44) or not typical (#9, #23, #37, #49) facial RSTS characteristics. Numbers in this figure correspond to those in Table I. Note the variation in main RSTS signs such as arching of eyebrows, down-slanting of palpebral fissures, positioning of the columella below the nasal alae, convexity of the nasal ridge, and micrognathia. B: Distal limb anomalies in patients with *EP300* mutations. Note the variation in broadening of the thumbs but absence of radial deviation, and variation in broadening of end-phalanges of fingers. Also the big toes can be normal or broad, and long or short. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

TABLE II. Summary of Main Findings in Present Series of RSTS Individuals With EP300 Mutations Compared to RSTS Individuals With CREBBP Mutations Reported in Literature

	EP300 (n = 52)		CREBBP[1] (n = 308)		*P*-value[2]
Prenatal growth retardation	42%	20/48	25%	52/204	<0.05*
Preeclampsia	23%	12/52	3%	2/59	<0.01*
Postnatal growth retardation	66%	33/50	75%	160/214	NS
Microcephaly (OFC<3rd centile)	87%	45/52	54%	77/143	<0.00005****
Hypertrichosis	51%	23/45	76%	93/122	<0.005**
Arched eyebrows	65%	34/52	85%	71/84	<0.05*
Long eyelashes	90%	44/49	89%	75/84	NS
Downslanted palpebral fissures	56%	29/52	79%	208/263	<0.001**
Epicanthal folds	15%	8/52	44%	92/208	<0.0005***
Convex nasal ridge	44%	23/52	81%	225/278	<0.00005****
Columella below alae nasi	92%	48/52	88%	195/222	NS
Grimacing smile	47%	21/45	94%	99/105	<0.00005****
Highly arched palate	67%	30/45	77%	160/208	NS
Micrognathia	42%	22/52	61%	131/214	<0.05*
Low-set ears	27%	14/52	44%	72/164	<0.05*
Broad thumbs	69%	36/52	96%	277/290	<0.00005****
Angulated thumbs	2%	1/51	49%	135/273	<0.00005****
Broad halluces	81%	42/52	95%	221/233	<0.005**
Intellectual disability	94%	48/51	99%	250/253	NS
Severe	7%	2/29	36%	33/92	<0.005**
Moderate	31%	9/29	48%	44/92	NS
Mild	62%	18/29	14%	13/92	<0.00005****
Epilepsy	10%	5/50	25%	32/126	<0.05*
Autism/autistiform behavior	25%	12/49	49%	51/105	<0.005**
Cardiovascular anomalies	26%	12/47	35%	64/181	NS
Urinary tract anomalies	24%	11/45	28%	35/124	NS
Scoliosis	25%	12/49	18%	34/184	NS
Obesity	39%	20/52	29%	42/143	NS
Keloids	10%	5/49	23%	39/168	NS

[1]Figures derived from Schorry et al. [2008]; Riscioni et al. [2015]; Spena et al. [2015]; Wincent et al. [2015] unpublished observations (D.L.; R.C.H.).
[2]Fisher's exact test with $P < 0.05$ considered significant *.

of the sphenoid bones. A pectus excavatum was present in five patients. Syringomyelia, sacral lipoid meningocele, and spina bifida occulta occurred each once. In 32 cases (60%), hand radiographs were available and showed one case with duplication of distal first phalanx, enlarged first phalanx in 63% (19/30), and brachytelephalangy in 22% (7/32) cases. Pilomatricomas were found in 11% (5/45) of the patients, multiple nevi in two patients, and tuberous angioma in one patient. The phenotype of the three patients (cases 25, 26, and 49) in whom the *EP300* mutation was detected by exome sequencing using a targeted approach for genes known to cause intellectual disability, did not seem to differ markedly from the patients in whom clinically the diagnosis RSTS was considered.

Development

Oligohydramnios was observed in one case with premature birth at 34.5 weeks of gestation. Additional ultrasonographic signs were choroid plexus cysts, agenesis of left kidney and enlarged aorta. Polyhydramnios was not observed. In addition to the 12 mothers with preeclampsia there were five who had hypertension or marked hypertension during pregnancy. None of the mothers had preeclampsia in an earlier or subsequent pregnancy (no exact number known). Gestational duration varied markedly from 31 to 41.7 weeks. Intra-uterine growth retardation was observed in 42% (20/48) and postnatal growth retardation in 60% (30/50) cases.

Age of first words was delayed in 57% cases (24/42) with only a few sentences at age 8 in one case associated with a severe intellectual disability. Delayed age of walking (after 18 months of age) was observed in 79% cases (34/43). Absence of intellectual disability was observed in 6% cases (3/51), aged 30, 39, and 44 years.

Natural History

The general health of the patients was good. The delayed development and especially the variable behavioral problems were the main concerns for the families. Recurrent upper airway infections were reported but it remains uncertain whether this was more frequent than in the general population. Gastro-esophageal reflux was specifically mentioned in six patients. One patient developed breast

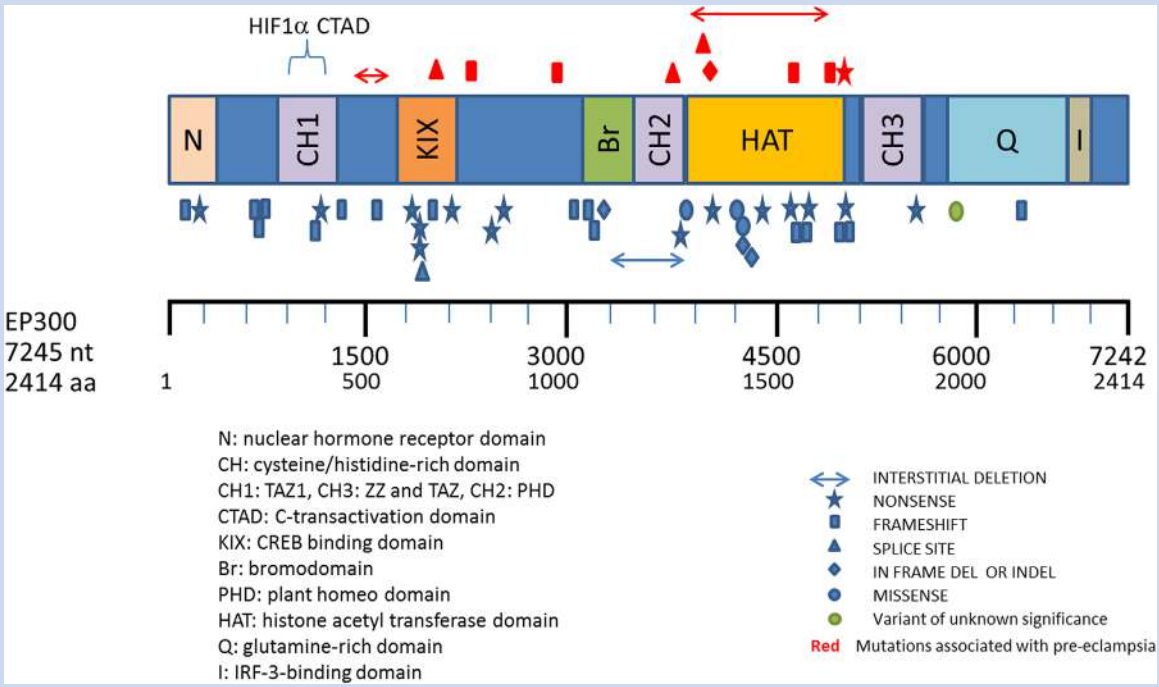

FIG. 2. Distribution of the 52 *EP300* mutations and interstitial deletions (51 different alterations). Symbols represent the mutation types as indicated. All the mutations found in patients mothers presenting with pre-eclampsia are shown in red above the schematic representation of P300 [adapted from Freedman et al., 2002; Dancy and Cole, 2015]. From the P300 interactome, only the interaction of CH1 with the hypoxia inducible factor 1 (HIF1) trans-activation domain is indicated because of the role of HIF1 in pre-eclampsia. The inherited missense change is labeled as a variant of unknown significance (VUS). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

cancer at age 55 years; she did not have keloids. Another patient had recurrent marked anemia from the age of 30 years on, and at 36 years was found to have sarcoidosis-like lung changes that responded reasonably well to steroids. An additional patient had persistent hypokalemia for which no cause was found, and another patient was diagnosed with ichthyosis.

Genotype

We identified 49 heterozygous point mutations throughout the gene (Fig. 2) including 14 different nonsense and 23 frameshift mutations, four in-frame del or indels (#10, #36, #44, #49), three de novo missense variants located in exon 23 (#5), 26 (#4), and 27 (#11), one inherited missense variant in exon 31 (#33), and four intronic variants predicted to exert splicing effect in introns 9 (#48), 10 (#52), 21 (#28), and 24 (#13). The same nonsense mutation p.Arg626* was found in patients #26 and #46 coming from separate countries and referred to two different laboratories. Exon 2 mutation p.Ser35Tyrfs*12 already identified by exome sequencing in a boy presenting with a Cornelia de Lange-like syndrome [Woods et al., 2014] was also found in a boy referred to us as a mild RSTS with some resemblance to Cornelia de Lange syndrome (unfortunately no consent for publication of facial pictures could be obtained). All other mutations have not been reported before. The three de novo missense variants were all predicted as deleterious according to SIFT (score: 0), probably pathogenic with PolyPhen 2 (HumVar score >0.98), and MutationTaster (disease causing, *P*-value: 1). In addition, the variant c.3857A>G. (p.Asn1286Ser) found in patient #5 (see pictures Fig. 1) was predicted to create a splice site within exon 23 displaying exactly the same scoring as the natural one (five software programs via Alamut interface). This new donor site may lead at least partially to an in frame deletion of six residues at the N-terminal end of the HAT domain. However, no RNA source was available to confirm any splicing effect for this exonic variant. The splice variant in intron 10 affected a consensus splice site (c.2053+1) whereas the variants in introns 9, 21, and 24 (respectively, c.1878-12A>G, c.3728+5G>C, c.4026-9A>G) were outside any donor or acceptor splice site. A de novo acceptor site could appear in intron 24 along with a drastic decrease in the natural site scores, leading to a premature stop codon as already shown using transcript analysis for c.1878-12A>G in intron 9 [Negri et al., 2016]. Exon skipping was predicted for c.3728+5G>C resulting in an in frame deletion of exon 21 and for c.2053+1G>C resulting in a premature stop codon. Gene dosage anomalies in the three remaining patients were interstitial deletions.

There was no evidence for mosaicism in any patient. There was no recurrence within a family. In all families in which parents

were tested the mutation in the affected child could not be detected in a parent, except in patient 33 with a missense mutation well outside the HAT domain, at the C-terminal end of *EP300*, in whom the unaffected father was found to have the mutation as well [Wincent et al., 2015]. There was no sign of mosaicism in the father, and the patient did not have a variant in *CREBBP*. The phenotype of the patient did not differ from the phenotype of the other patients except for the presence of angulated thumbs.

DISCUSSION

We present here the results of an international collaboration aimed at providing more information of the consequences of *EP300* mutations for patients. Until now, 26 patients with an *EP300* mutation have been reported in the literature to which we add now 42 further patients and provide an update of 10 of the earlier reported patients.

Phenotype

The phenotype in the present cohort of patients with *EP300* mutations shows in all patients clues pointing to RSTS. Indeed, except for three patients all mutations have been detected by targeted analysis of *EP300*, often after first having excluded detectable *CREBBP* mutations. Also the phenotype of the three patients in whom the *EP300* variant was detected by panel exome sequencing because of their intellectual disability showed in retrospect resemblance to the RSTS phenotype, although possibly to a lesser extend compared to those detected by targeted analysis. However, numbers are too small to draw any conclusion about this. However, the characteristics in *EP300* mutated patients are usually less marked compared to patients with *CREBBP* mutations (Table II). The major facial characteristic in RSTS is considered to be the grimacing smile which is almost universally present in patients with *CREBBP* mutations but only in half of the *EP300* mutated patients. The same holds for the facial morphology of eyebrows, palpebral fissures, nasal ridge, chin, and ears. The exception is the positioning of the columella below the nasal alae which is as frequent in individuals with an *EP300* mutation as in those with a *CREBBP* mutation. The limb characteristics are more similar to those in *CREBBP* mutated patients although still somewhat less frequent. Only angulation of thumbs and halluces is extremely uncommon in patients with *EP300* mutations. There is no major difference in malformations of internal organs between both groups of patients as in both about one-quarter of the patients have such malformations.

Growth is disturbed prenatally and postnatally, both with respect to height and brain growth. The prenatal growth retardation is somewhat more expressed in patients with *EP300* mutations, which may be associated with the frequent pre-eclampsia (6 of the 12 patients with pre-eclampsia were small at birth). Adult height varied from 151 to 159 cm in females and 158–176 cm in males. Brain growth, evident in skull circumference, is more disturbed in the *EP300* mutated patients compared to those with a *CREBBP* mutation. This is remarkable as cognitive functioning is typically higher in *EP300* mutated patients. More than half of them have a mild intellectual disability and severe intellectual disability is rare, while the latter occurs in about one-third of *CREBBP* mutated patients and less than 15% have mild intellectual delay. Several adults with *EP300* mutation have been able to live independently in a somewhat sheltered environment. This difference is mirrored in *p300+/−* mice who show similar but less marked deficits compared to *cbp+/−* mice [Viosca et al., 2010]. Behavior problems are frequent in *EP300* mutated patients. In a quarter of the patients autism or autism-related traits are present. It has been stated that especially mutations in the CH1 domain in *CREBBP* may lead to autism [Zheng et al., 2016] but this was not confirmed here in the *EP300* mutations as the two patients with a mutation in this domain showed no autism. Also other behavioral problems occurred frequently of which obsessive-compulsive behavior was the most significant, but anxieties and self-injurious behavior were also common. Less frequently hyperactivity, aggression, and stereotypies were noticed. This behavior resembles the behavior in *CREBBP* mutated individuals [Galera et al., 2009; Yagihashi et al., 2012; Waite et al., 2015], especially with respect to the obsessive-compulsive behavior and occurrence of anxieties. Likely, the behavior can be explained directly by the disturbed function of the P300 protein [Nonaka et al., 2014].

Genotype

All type of mutations do occur in patients with an RSTS phenotype and an *EP300* variant. Truncating mutations and small rearrangements represent 86% of *EP300* anomalies. Twenty-seven percent (14/52) of all the mutations were found in the six exons encoding the histone acetyl-transferase(HAT) domain, covering 12% of the *EP300* coding sequence. Considering both de novo missense and in-frame del or indels, the seven mutations without truncating effect were located in CH2 domain and at the N-terminal half of the HAT domain. The three de novo *EP300* missense variants were located in the HAT domain, spanning amino acid residues 1284_1673 [Ogryzko et al., 1996]. There is one additional missense mutation, inherited from a healthy father, located well outside the HAT domain, in exon 31 of *EP300* [Wincent et al., 2015]. It remains uncertain whether this mutation is causative for the phenotype or not. With the increasing use of targeted exome, sequencing checking for variants in genes known to cause intellectual disability, the number of variants in such genes in individuals without the classical phenotype reported in literature is also increasing. Recently, we found 11 individuals with a variant in *CREBBP* in a small region of exon 30/31, without known function, who shared partly their phenotype [Menke et al., 2016]. The likelihood this has occurred by chance is small but pathogenesis remains uncertain at present. Similarly, the present change in exon 31 of *EP300* in the patient reported by Wincent et al. [2015] and updated here, may prove later on to be causal as well, although one should also take into account that there is no or only partly an association between the P300 variant and the phenotype in this family. Combining patients with variants of uncertain meaning in databases, together with a detailed phenotype, will be essential to determine their causal role [Hennekam and Biesecker, 2012].

Genotype–Phenotype Correlations

We have been unable to detect a statistically significant correlation between the phenotype and the location of the mutations, which can be true or may be caused by the small cohort size. Three out of the four intronic splicing mutations were associated with pre-eclampsia and the fourth one with hypertension in the patient's mother, but this can still be just coincidence. It is of note that the three patients with missense mutations in the HAT domain of *EP300* had the typical RSTS facial morphology and cognition. If this correlation would remain present in larger series this would indicate a difference with RSTS individuals with *CREBBP* mutations as the classical phenotype in *CREBBP* mutations can be found in truncating mutations anywhere in *CREBBP* and mutations in the HAT domain in *CREBBP* can have both the classical RSTS morphology (including deviated thumbs) and cognition but can also lead to a mild phenotype [Schorry et al., 2008; Spena et al., 2015; unpublished data]. The HAT domain is highly conserved between the two paralogs. It is tempting to postulate that missense mutations affecting the HAT domain of both *CREBBP* and *EP300* lead to the typical RSTS morphology and intellectual disability but this needs more data before it can be proven. We did not find a statistically significant difference in frequency of mutations in *CREBBP* and *EP300* depending on their site. The number of mutations in exon 31 is somewhat low compared to the size of exon 31 but this is not statistically significant. Still one cannot exclude that mutations in exon 31 cause a phenotype that is not studied for *EP300* variants as the phenotype is not known to be caused by *EP300* mutations.

Pre-Eclampsia

In the general population pre-eclampsia occurs in 5–8% of pregnancies [Steegers et al., 2010]. In the present cohort 23% of the pregnancies were complicated by pre-eclampsia and another 10% had marked hypertension. We have been unable to find another factor in the literature that predicts occurrence of pre-eclampsia with such high frequency. We (D.L; R.C.H.) have set-out a specific questionnaire in the support groups for families with a child with RSTS caused by a *CREBBP* mutation in the Netherlands and France, and 2 of 52 families indicated that preeclampsia had occurred during the pregnancy of the child with RSTS. This indicates that the risk of pre-eclampsia is likely to correlate specifically with *EP300* mutations.

Pre-eclampsia is a disorder of which many hypotheses exist regarding its cause and pathogenesis ("the disease of theories") [Lindheimer and Umans, 2006; Steegers et al., 2010]. It was already known that as well as maternal and paternal factors fetal factors may also play a role [Petry et al., 2014], such as a fetus having Beckwith–Wiedemann syndrome, if caused by a *CDKN1C* mutation [Romanelli et al., 2009]. Several case reports of RSTS individuals with *EP300* mutations and pre-eclampsia have been published and the causes of the EP300 pre-eclampsia have been discussed elsewhere [Van Uitert et al., 2015].

Placental transcriptome analysis in 116 pre-eclamptic pregnancies compared to 139 normotensive pregnancies and subsequent pathway analysis demonstrated a correlation with the hypoxia/HIF1A pathway but also with CREBBP/P300 [Van Uitert et al., 2015]. In a Finnish study of gene expression profiles of placentae from pregnancies complicated by pre-eclampsia a large number of genes was upregulated [Kaartokallio et al., 2015] but CREBBP and EP300 were however not upregulated. This may be caused by the unusual population characteristics of the Finnish population [Dr Tea Kaartokallio, personal communication 2016]. In our cohort, all *EP300* mutations associated with pre-eclampsia in mothers are truncating except the intronic mutation that could lead to exon 21 skipping and the in-frame deletion in exon 25. Previous analysis of *EP300* transcripts in two patients bearing mutations associated with hypertension in their mothers, c.1879-12A>G and p.Lys1277* showed the presence of both the wild-type and the aberrant transcripts supporting protein truncation as the main functional consequence rather than NMD [Negri et al., 2016]. The fact that all mutations associated with pre-eclampsia in mothers are located downstream of the CH1 domain of the protein suggest that this P300 domain, which is known to interact with HIF1, was not disrupted although we cannot exclude that the protein is degraded via nonsense-mediated decay.

Many, especially immunologic, hypotheses can be formulated to explain an associationbetween *EP300* mutations in fetuses and pre-eclampsia in their mothers, but until now all remain unproven. The present high frequency of pre-eclampsia in mothers carrying a fetus with an *EP300* mutation does urge for further functional studies.

We conclude that *EP300* mutations can cause a phenotype that to a great extent resembles the phenotype described in Rubinstein–Taybi syndrome due to *CREBBP* mutations. The facial characteristics are less marked, however, with the exception of the low-hanging columella which is as frequent in *EP300* mutated individuals as in *CREBBP* mutated individuals. The same holds for the limb defects, except for radially deviated thumbs which occur only in a single *EP300* mutated individual in whom the causality of the mutation remains uncertain. All levels of cognitive problems were observed but in general the level of cognitive function is higher in *EP300* mutated individuals. No correlation was found between the nature or site of *EP300* mutations and the phenotype. Being pregnant with a child with an *EP300* mutation is at present the highest known risk factor to develop pre-eclampsia, and further functional studies to explain this are urgently needed.

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