Persistent hyperinsulinaemic hypoglycaemia in children with Rubinstein–Taybi syndrome

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Abstract

Objective: Genetic aetiology remains unknown in up to 50% of patients with persistent hyperinsulinaemic hypoglycaemia (HH). Several syndromes are associated with HH. We report Rubinstein–Taybi syndrome (RSTS) as one of the possible causes of persistent HH. Early diagnosis and treatment of HH is crucial to prevent hypoglycaemic brain injury.

Design: Four RSTS patients with HH were retrospectively analysed.

Methods: Genetic investigations included next-generation sequencing-based gene panels and exome sequencing. Clinical characteristics, metabolic profile during hypoglycaemia and treatment were reviewed.

Results: Disease-related *EP300* or *CREBBP* variants were found in all patients, no pathogenic variants were found in a panel of genes associated with non-syndromic HH. Two patients had classic manifestations of RSTS, three had choanal atresia or stenosis. Diagnosis of HH varied from 1 day to 18 months of age. One patient was unresponsive to treatment with diazoxide, octreotide and nifedipine, but responded to sirolimus. All required gastrostomy feeding.

Conclusions: Given the rarity of RSTS (1:125 000) and HH (1:50 000), our observations indicate an association between these two conditions. We therefore recommend that clinicians should be vigilant in screening for HH in symptomatic infants with RSTS. In children with an apparent syndromic form of HH, RSTS should be considered in the differential diagnosis.

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Introduction

Hyperinsulinaemic hypoglycaemia (HH) is a heterogeneous disorder characterized by recurrent episodes of hypoglycaemia due to the dysregulated release of insulin from pancreatic beta cells despite low blood glucose concentrations (1). Although a rare disorder, HH is the most common form of persistent hypoglycaemia in the neonatal period and early infancy (2).

Recurrent hypoglycaemic insults to the developing brain are associated with serious lifelong complications such as permanent brain injury and seizures (3, 4, 5). Therefore, early recognition and treatment of HH is the cornerstone for improving neurodevelopmental outcomes (6). HH represents a group of clinically, genetically and morphologically heterogeneous disorders. It can be

caused by pathogenic variants in key genes regulating insulin secretion but has also been reported as a feature in at least 20 different rare genetic syndromes, which include overgrowth syndromes such as Beckwith–Wiedemann syndrome (BWS) and Sotos syndrome, postnatal growth failure syndromes such as Costello and Kabuki syndrome, congenital disorders of glycosylation and chromosome abnormalities such as Turner syndrome and Patau syndrome (7, 8, 9, 10). The complexity of some syndromic conditions may lead to difficulty in recognising a co-existing diagnosis of HH. This may delay the diagnosis of HH and further impair the neurodevelopmental outcomes of these children. Moreover, an early genetic diagnosis is important to guide medical management and provide information on prognosis and recurrence risk.

We here describe the association of Rubinstein–Taybi syndrome (RSTS) and persistent HH, and systematically summarize the clinical course of four affected patients. RSTS is a rare developmental disorder characterized by postnatal growth deficiency, microcephaly, typical facial appearance, distal limb abnormalities and a broad range of cognitive impairment. Comorbidities such as cardiovascular or urinary tract anomalies and epilepsy may be present in up to 30% of RSTS patients (11). Pathogenic variants in two genes are known to cause RSTS, i.e. those of the cAMP response element-binding protein (CREB)-binding protein (*CREBBP*-encoding CBP) and of E1A-binding protein P300 (*EP300*-encoding P300). The majority of cases (60–70%) are caused by haploinsufficiency of the *CREBBP* gene. Heterozygous variants disrupting the paralogous gene *EP300* are found in up to 10% of patients. The genetic cause is unknown in approximately 20–30% of cases (11, 12, 13, 14). Although single reports of hypoglycaemia in RSTS patients have been published, RSTS has not yet been recognized as a syndromic cause of persistent HH (15, 16, 17). Wyatt *et al.* reported a case of transient HH in a small for gestational age (SGA) newborn with RSTS that required a glucose infusion at twice the normal endogenous glucose production rate of SGA infants to maintain euglycemia. A genetic diagnosis for this patient is not available. More recently, Lopez *et al.* and Costain *et al.* reported on individuals with RSTS and HH due to pathogenic variants in *EP300* (deletion of exons 12–21 of *EP300*; heterozygous *de novo* missense variant in the HAT domain of *EP300*).

We review the clinical course and genetic findings of four RSTS patients with HH, providing additional information on the clinical characteristics and metabolic phenotype of these patients and increasing the evidence for an association between RSTS and HH.

Patients and methods

Four patients with genetically confirmed RSTS and HH were retrospectively analysed. Written informed consent for publication of their clinical details was obtained from all patients or their respective guardians. Genetic investigations were either targeted for pathogenic variants in *EP300* and *CREBBP* when RSTS was clinically suspected or untargeted as part of the deciphering developmental disorders (DDD) study when developmental delay was the predominant feature. When HH and/or other clinical features were prevailing and targeted testing for specific syndromes was negative, next-generation sequencing (NGS)-based gene panels or exome sequencing were applied. Pathogenic variants in genes associated with congenital hyperinsulinism (CHI) were excluded in all cases other than case 2 where targeted testing of *CREBBP* was arranged due to the clinical suspicion of RSTS. Sequence variants were classified according to ACMG criteria (18). Metabolic profiling included the measurement of insulin and c-peptide, free fatty acids and blood or urinary ketones during hypoglycaemia ≤ 2.7 mmol/L. Hypoglycaemia was defined as hypoketotic hypoglycaemia when beta-hydroxybutyrate level was < 1.8 mmol/L or urine ketones were negative. Low free fatty acids (FFA) were defined as FFA < 1.7 mmol/L (19). Initial glucose requirement is defined as the amount of carbohydrates initially required to prevent hypoglycaemia < 3.5 mmol/L.

Results

Clinical characteristics, metabolic profile, genetic investigations and treatment regime of all individuals are summarized in Table 1.

Case 1

Individual 1 (P1) was born at 38-weeks gestation, with a birth weight of 2600 g (-1.2 SDS). She had subtle facial features out of keeping for age and ethnicity but not at that time suggestive of an underlying syndromic diagnosis. Other clinical features included bilateral choanal atresia requiring nasal stent insertion in the neonatal period, developmental delay and gastro-oesophageal reflux disease (GORD) with unsafe swallow resulting in silent micro-aspirations. She also had episodes of tachycardia and bradycardia with normal sinus rhythm on 24-h ECG recordings and small pericardial effusion with structurally normal heart on echocardiogram. MRI brain

Table 1 Clinical characteristics, metabolic profile, genetic investigations and treatment regime of four RSTS patients with persistent HH.

	Patient 1	Patient 2	Patient 3	Patient 4
Gestational age (w)	38	38	38	36
Birth weight (g)	2600	2660	2985	2400
Sex	Female	Male	Female	Female
Dysmorphic features	mild non-specific dysmorphic features	typical facial appearance of RSTS	typical facial appearance of RSTS	partial facial appearance of RSTS
Other clinical features	Bilateral choanal atresia, GORD, episodes of tachykardia and bradycardia, dermoid cyst at orbital roof/sphenoid wing	GORD, undescended testes, laryngomalacia, cataract, PDA	Choanal stenosis, tricuspid atresia type 1c, short stature	Bilateral choanal atresia, GORD, unilateral multicystic dysplastic kidney, ASD, dilatation of the asc. aorta, mild conductive hearing loss, recurrent small hymenal cyst, query Müllerian anomaly
Developmental delay	Yes	Yes	Yes	Moderate expressive speech delay
Age at diagnosis of RSTS	2.38 years	<1 months	4.5 years	1.58 years
Age at diagnosis of HH	1.54 years	Day 1 of life	Day 5 of life	1.42 years
Metabolic profile during hypoglycaemia				
Glucose, mmol/L	2.3	1.6	2.2	2.1
Insulin, pmol/L	27	36	154	39
C-peptide, pmol/L	N/A	612	n.d.	n.d.
Hypoketotic	Yes	Yes	Yes	Yes
Free Fatty acids	Low	Low	Low	Low
Pathogenic mutation	Heterozygous <i>de novo</i> missense mutation c.3764A>G, p.(His1255Arg) in <i>EP300</i>	Heterozygous frameshift mutation c.1044delT, p.(Glu349Lysfs*5) in <i>CREBBP</i>	Heterozygous <i>de novo</i> missense mutation c.4505C>T, p.(Pro1502Leu) in <i>EP300</i>	Heterozygous <i>de novo</i> missense mutation c.4783T>G, p.(Phe1595Val) in <i>EP300</i>
Additional investigations related to HH		none specific		
Scan	18F-DOPA PET/CT: diffuse uptake			
Mutation in known CHI genes	None*		None*	None*
Initial glucose requirements	n.d.	12 mg/kg/min	>8 mg/kg/min	n.d.
HH medications (initial dosage)				
DZX	Unresponsive	Response unclear due to parental malcompliance	Responsive (<5 mg/kg/day)	Responsive (10.6 mg/kg/day)
NIF	Unresponsive	Unresponsive		
OCT	Unresponsive			
Sirolimus	Responsive (5 mg/m ² /day)			
HH medication-related severe side effects	Sirolimus: sepsis	DZX: fluid overload	None	DZX: fluid overload
PEG feedings	Yes	Yes	Yes	Yes
Feeding regime at discharge (after diagnosis of HH)				
Feeds	Daytime bolus			Daytime bolus
PEG feeds	Continuous overnight	Continuous day and night	Continuous overnight	Continuous overnight
Age at patient's last clinic visit (years)	3.9	7.87	4.8	4
Current management of HH				
Feeds	Overnight PEG feeds	Normal feeding, acarbose because of postprandial HH		Bolus PEG feed at bedtime
Fasting tolerance	11 h		11–15 h	
Medication			DZX: 7.5 mg/kg/day	DZX: 6 mg/kg/day

*Including ABCC8, KCNJ11, GCK, GLUD1, HADH, HNF1A, HNF4A, SLC16A1, UCP2.

ASD, atrial septal defect; DZX, diazoxide; GORD, gastro-oesophageal reflux disease; NIF, nifedipine; OCT, octreotide; PDA, patent ductus arteriosus; PEG, percutaneous gastrostomy.

showed normal intracranial appearances including the hypothalamic pituitary axis. A well-defined cystic lesion related to the left sphenoid wing or orbital roof, consistent with a dermoid cyst was seen. Electromyography and nerve conduction studies showed minor abnormalities of peripheral nerve conduction.

She had recurrent episodes of hypoglycaemia from the age of 1.5 years and had generalized tonic clonic seizures following an episode of hypoglycaemia. Further biochemical investigations confirmed the diagnosis of HH (Table 1). She was then commenced on diazoxide at the dose of 5 mg/kg/day and chlorothiazide 7 mg/kg/day. A percutaneous gastrostomy was also inserted, as part of the management of HH, along with severe GORD and unsafe swallow. She was fed with frequent bolus feeds during the daytime and continuous gastrostomy feeds overnight. However, she continued to have episodes of hypoglycaemia and was transferred to a specialist centre for further management of HH. Genetic testing was negative for known non-syndromic causes of HH (*ABCC8*, *KCNJ11*, *GCK*, *GLUD1*, *HADH*, *HNF1A*, *HNF4A*, *SLC16A1*). ¹⁸F-DOPA-PET/CT scan suggested diffuse uptake of ¹⁸F-DOPA. The patient was unresponsive to maximum doses of diazoxide, octreotide and nifedipine. She was tried on sirolimus (mTOR inhibitor) therapy (maximum 5 mg/m²/day), to which she demonstrated a good response, but later developed severe systemic infection (sepsis). Consequently, sirolimus was stopped at the age of 2.6 years and she was managed with an intensive feeding regime. Thickened liquids and pureed food were gradually introduced into her diet.

This individual was recruited to the DDD project (20, 21) to try to understand the cause of her difficulties and a diagnosis of RSTS was made at 2.4 years following the identification of a likely pathogenic variant in *EP300* c.3764A>G p.His1255Arg.

At her last clinic review at the age of 3.9 years she successfully tolerated 4–6 hourly overnight gastrostomy feeds and on demand feeds during daytime (to provide adequate calories). Fasting test revealed a fasting tolerance of 11 h (blood glucose 2.9 mmol/L, undetectable insulin <14 pmol/L).

Case 2

Individual 2 (P2) was born at 38 weeks gestation to non-consanguineous parents with a birth weight of 2660 g (−1.2 SDS). P2 was noticed to have poor feeding on day 1 of life and on day 2 he had a generalized seizure associated with low blood glucose of 0.9 mmol/L. He was admitted

to the neonatal unit and hypoglycaemia was managed by regular feeds and intravenous fluids. He was noted to have feeding difficulties, including choking episodes, leading to an apnoeic episode and was diagnosed with GORD and started on anti-reflux medications in the first week of life. Feeds were subsequently changed to an amino acid-based formula due to a family history of cow's milk protein intolerance.

He was also noted to have coarse facial features, broad and angulated thumbs and broad halluces. Other findings included bilateral undescended testes, mild laryngomalacia, left anterior polar cataract and a patent ductus arteriosus which closed spontaneously. Due to a history of seizure-like episodes, he had a brain MRI and CT, which showed some non-specific white matter changes and delayed myelination. Electroencephalogram (EEG) did not show the presence of epileptiform activity.

He continued to have intermittent episodes of hypoglycaemia and his hypoglycaemia screen showed hypoketotic HH (blood glucose 1.6 mmol/L, insulin 36 pmol/L, C-peptide 612 pmol/L, negative urine ketones). He had high glucose requirements (12 mg/kg/min) in order to maintain euglycaemia. Subsequently, he was started on diazoxide on day 38, but this was stopped a few days later due to signs of fluid overload. On day 46, the patient was transferred to a specialist centre for the management of HH. He was managed on regular feeds and at the age of 2.5 months, he fasted for 6 h and was managed on regular high calorie feeds (blood glucose 4.2 mmol/L, undetectable insulin <14 pmol/L). He had persistent vomiting on continuous feeds and video fluoroscopy showed poor suck and unsafe swallow. A barium study confirmed GORD and he underwent a Nissens fundoplication at 8 months of age. He developed post prandial hypoglycaemia with an inappropriate presence of insulin following OGTT (glucose 2.5 mmol/L, insulin 78.5 pmol/L). This suggested that the mechanism of his hypoglycaemia is related to surges in insulin secretion in response to glucose ingestion (carbohydrate containing meals). He was treated with continuous feeds via gastrostomy and slow bolus feeds.

He was given another trial of diazoxide (up to 10 mg/kg/day) along with 2-hourly bolus feeds. However, he continued to have hypoglycaemic episodes and a decision was made to stop the diazoxide as parents were not keen to continue due to its side effects of hypertrichosis. He also had a trial of nifedipine but did not show any improvement in his blood glucose levels. He was then discharged home on 24-h continuous feeds. He was gradually tried on small bolus feeds at the age of 4.0 years

and was commenced on acarbose (25 mg three times per day) with his bolus feeds. He is currently 7.87 years, on acarbose (25 mg three times per day) and on demand feeding regime. He continues to have occasional episodes of post prandial hypoglycaemia with a good fasting tolerance of 18 h with no hypoglycaemia at the end of the fast (blood glucose 4.1 mmol/L, undetectable insulin <14 pmol/L, FFA 1.06 mmol/L, beta-hydroxybutyrate 1.17 mmol/L).

His genetic investigations confirmed a heterozygous pathogenic variant c.1044delT, p.Glu349Lysfs*5 in *CREBBP* which confirmed the diagnosis of RSTS.

Case 3

The third individual (P3) was born at 38 weeks of gestation to healthy, non-consanguineous parents. Her birth weight was 2985 g (−0.45 SDS), length 47 cm (−1.48 SDS) and head circumference 31.8 cm (−1.92 SDS). Cleft palate, dysmorphic features and central cyanosis were noted at birth requiring intensive care treatment. Further diagnostic evaluations revealed tricuspid atresia type Ic that was surgically managed, later resulting in Fontan circulation.

Since day 5 of life, recurrent hypoglycaemia was observed requiring >8 mg/kg/min carbohydrates. During hypoglycaemia (<2.8 mmol/L), repeated evaluations showed inappropriate insulin secretion (154 and 249 pmol/L), suppressed ketones and low FFA, thus leading to the diagnosis of HH. At 6 weeks of age, diazoxide treatment (<5 mg/kg/day) was initiated leading to improved glycaemic control. However, for unclear reasons diazoxide was not titrated above 5 mg/kg/day, although carbohydrate requirements were still increased. At hospital discharge at 6 months of age, HH was managed with low-dose diazoxide (2.5 mg/kg/day), frequent daytime feeds and continuous percutaneous gastric tube (PEG) feeding overnight.

At first admission to a HH specialist service at 4.3 years, body weight was 13.3 kg (−1.79 SDS), length 90.4 cm (−3.2 SDS) and head circumference 45.5 cm (−3.7 SDS). Facial features such as a beaked nose, arched eyebrows, long eyelashes, left ear auricular dysplasia, long philtrum with thin upper lip, ptosis, intermittent strabismus and choanal stenosis were noted, as well as coarse wrists and psychomotor difficulties. Glycaemic control was achieved by low-dose diazoxide treatment (2.2 mg/kg/day) in addition to giving daytime meals every 4–5 h and continuous overnight feeding via PEG (stepwise decreased to 1.4 mg/kg/min glucose). After pausing diazoxide for

7 days, formal testing revealed a fasting tolerance of less than 6 h (blood glucose 2.8 mmol/L, insulin 26 pmol/L, suppressed ketones and FFA). Therefore, diazoxide treatment was commenced again and gradually increased to 7.5 mg/kg/day, leading to stable blood glucose levels >3.9 mmol/L and a fasting tolerance of 11–15 h without the need for further continuous overnight gastrostomy feeding.

NGS excluded disease-causing pathogenic variants in genes known to cause HH (*ABCC8*, *ALG3*, *GCK*, *GLUD1*, *HADH*, *HK1*, *HNF1A*, *HNF4A*, *HRAS*, *INSR*, *KCNJ11*, *KCNQ1*, *KDM6A*, *KMT2D*, *MAFA*, *MPI*, *NSD1*, *PAX6*, *PGM1*, *PMM1*, *PMM2*, *SLC16A1*, *TRMT10A*, *UCP2*) or cardiofaciocutaneous syndromes (*BRAF*, *KRAS*, *NRAS*, *PTPN11*, *RAF1*, *RIT1*, *SOS1*). Genetic testing of other syndromic conditions identified a heterozygous *de novo* missense variant in *EP300* (c.4505C>T p.(Pro1502Leu)), classified as likely pathogenic according to ACMG criteria (18), confirming the diagnosis of Rubinstein–Taybi syndrome.

Case 4

The fourth individual (P4) has been described previously (16). She was born at 36 weeks of gestation to healthy, non-consanguineous parents of English (mother) and Balkan (father) descent. Her birth weight was 2400 g (−0.79 SDS), length 48 cm (−0.15 SDS) and head circumference 33 cm (−0.2 SDS). Bilateral choanal atresia required immediate intensive care treatment and nasal stenting at day 16 of life. Right multicystic dysplastic kidney had been diagnosed prenatally and the right kidney was later found to be non-functional. At day 8 of life, a small ostium secundum atrial septal defect and an isolated dilatation of the ascending aorta were diagnosed, not requiring treatment.

At 5 months of age, poor oral intake and GORD required the insertion of a percutaneous gastric tube. At around 1.0 year when weaning her off gastrostomy feeding, parents first noticed excessive sleepiness as well as an episode with eye twitching suggestive of a seizure. At 1.4 years an episode of jitteriness and decreased responsiveness following a delayed feed prompted further diagnostic evaluations. Recurrent episodes of hypoketotic hypoglycaemia with inappropriately high serum insulin concentrations (39 pmol/L at blood glucose level of 2.1 mmol/L) led to the diagnosis of HH. Diazoxide treatment was initiated (10.6 mg/kg/day) and improved glycaemic control. Fasting tolerance at that time was approximately 5–6 h with no hypoglycaemia at the end of

the fast. Facial features included down-slanting palpebral fissures and microretrognathia, while some of the classic features of RSTS were absent. At 4.0 years of age HH is still managed with diazoxide treatment (6 mg/kg/day) and a bolus gastrostomy feed at bedtime. P4 has not required dose increases of diazoxide as she has grown. Apart from mild-to-moderate expressive speech delay, cognitive and motor development is age-appropriate. Currently, at 4 years, height is 100 cm (−0.7 SDS) and weight 15.8 kg (−0.18 SDS).

Genetic analysis revealed a heterozygous *de novo* missense variant in *EP300* (c4783T>G p.(Phe1595Val)) (16), classified as likely pathogenic according to ACMG criteria (18).

Discussion

Recent advances in molecular genetic testing have enabled the identification of novel genes linked with HH. To date, more than nine genes (*ABCC8*, *KCNJ11*, *GCK*, *GLUD1*, *HADH*, *HNF1A*, *HNF4A*, *SLC16A1*, *UCP2*) have been identified to be associated with monogenic congenital hyperinsulinism. However, in approximately 50% of clinically diagnosed patients with HH, genetic aetiology remains unknown, suggesting further unrecognised disease-associated genes (22, 23). The widespread availability of extended genetic testing has also facilitated the diagnosis of many individuals with syndromic conditions and an increased awareness of the association of HH with a number of these. BWS is a notable example but HH has also been reported as a feature in Sotos syndrome, Kabuki syndrome and others.

Early diagnosis of hypoglycaemia and appropriate treatment is essential to prevent neuroglycopenic brain damage (3, 5). This holds particularly true for patients with syndromic HH, as these children may already have an increased risk of seizures and mental retardation (9). We here systematically review the metabolic phenotype and clinical course of four RSTS patients with persistent HH.

Three of our four patients and one of the formerly reported individuals with RSTS and HH harbour a heterozygous disease-related variant in *EP300* (15). This is noteworthy, as *EP300* pathogenic variants only account for about 10% of RSTS patients and the clinical features are often more subtle compared to those with *CREBBP*-associated RSTS (11). Only one of our four patients (P2) harbours a heterozygous pathogenic variant in *CREBBP* which was associated with transient hyperinsulinism. However, he developed post prandial HH (dumping

syndrome). A possible cause of his post prandial hypoglycaemia was Nissen fundoplication surgery that was carried out due to severe GORD and feeding difficulties. Genetic testing was not available for the patient with transient HH and RSTS reported by Wyatt *et al.* (17). P2 and P3 exhibited typical clinical features suggestive of RSTS. Three of our patients were found to have choanal atresia or stenosis, which is not a commonly reported feature in RSTS.

Age at diagnosis of HH varied from day 1 and 5 of age in P2 and P3, respectively, to approximately 18 months of life in P1 and P4. The response to diazoxide treatment was variable. P1 was unresponsive to diazoxide, octreotide and nifedipine. This patient responded to sirolimus, but was ultimately managed with an intensive feeding regime due to sirolimus-associated sepsis. In contrast, P3 and P4 showed good response to diazoxide (5–10 mg/kg/day). In P2 there was an initial diazoxide response but this could not be assessed fully due to fluid overload. Subsequently diazoxide was trialled for dumping syndrome but was stopped after 10 mg/kg/day due to parental anxiety of its side effects. The patient reported by Wyatt *et al.* was responsive to moderate doses of diazoxide (10.9 mg/kg/day) that could already be discontinued after 14 days of treatment (17). All of our patients additionally required continuous gastrostomy feeding. At the age of now 4.8 years and 4 years P3 and P4 still require moderate doses of diazoxide (7.5 mg/kg/day and 6 mg/kg/day, respectively), while P1 and P2, aged 3.9 years and 7.9 years, maintain euglycaemic via overnight gastrostomy feeds (P1) or on demand feeds and acarbose (P2).

Given the rarity of both RSTS (1:125 000) and HH (1:40 000–50 000) (15, 24), the finding of at least four patients each suffering from both conditions clearly exceeds the expected frequency if both diagnoses were not linked in any way. However, the molecular mechanism underlying the association of HH with RSTS remains elusive. *EP300* and *CREBBP* are ubiquitously expressed and highly homologous genes, sharing >60% of protein sequence identity. They act as transcriptional co-activators with histone acetyltransferase activity. The *EP300* and *CREBBP* genes encode P300 and CBP, respectively, which hold similar functions in chromatin remodelling and are believed to participate in the activities of several different transcription factors (25). The molecular mechanism of RSTS therefore appears to be a global transcriptional disturbance due to defective genome-wide histone modification (26). Both P300 and CBP are required for normal pancreatic islet development, beta cell function and survival. However, mice lacking P300 or CBP in

pancreatic islets have reduced alpha and beta cell mass and become glucose intolerant due to hypoinsulinaemia (25). These findings in mice appear somehow contradictory to our observations. In all our patients hypoglycaemic episodes were associated with inadequately elevated levels of insulin strongly suggesting a mechanistic link. However, whether hyperinsulinism results from beta cell intrinsic pathways or from other factors such as paracrine glucagon signalling remains elusive. Since *Crebbp*- and *Ep300*-knockout mice both develop hypoinsulinaemia, overt or subclinical hyperinsulinism theoretically may progress to hypoinsulinism and glucose intolerance in RSTS patients. Clearly, additional mechanistic studies are required to elucidate the role of beta cell- and alpha cell-signalling in *CREBBP*- and *EP300*-associated HH.

In conclusion, although three of our four patients harbour a pathogenic variant in *EP300* rather than *CREBBP*, a definite genotype–phenotype correlation with respect to age at onset of HH and diazoxide responsiveness cannot be drawn from our data, due to small sample size. However, our report identifies persistent HH as a novel association of RSTS and expands the spectrum of HH syndromes. Our observations emphasize the need to carefully screen RSTS patients for hypoglycaemia, particularly those presenting with seizures or unspecific symptoms, to ensure early recognition of HH and the initiation of appropriate treatment. We furthermore recommend to include *EP300* and *CREBBP* in the genetic testing for patients with syndromic HH and clinical features suggestive for Rubinstein–Taybi syndrome.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

A Welters, R El-Khairi, P Shah and S Kummer: These authors contributed equally to this work as shared first authors and shared senior authors.

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