# Diagnosis and Management in Rubinstein-Taybi Syndrome: First International Consensus Statement

Didier Lacombe[1], Agnes Bloch-Zupan[2#], Cecilie Bredrup[3#], Edward B. Cooper[4#], Sofia Douzgou[5#], Sixto Garcia Minaur[6#], Hulya Kayserili[7#], Lidia Larizza[8#], Vanesa López-González[9#], Leonie A. Menke[10#], Donatella Milani[11#], Francesco Saettini[12#], Cathy A. Stevens[13#], Lloyd Tooke[14#], Jill A Van der Zee[15#], Maria M. Van Genderen[16#], Julien Van Gils[1#], Jane Waite[17#], Jean-Louis Adrien[18], Oliver Bartsch[19], Pierre Bitoun[20], Antonia H.M. Bouts[21], Anna M. Cueto-González[22], Elena Dominguez-Garrido[23], Floor Duijkers[24], Patricia Fergelot[1], Elizabeth Halstead[25], Sylvia A. Huisman[10,26], Camilla Meossi[11], Jo Mullins[27], Sarah M. Nikkel[28], Chris Oliver[29], Elisabetta Prada[11], Alessandra Rei[30], Ilka Riddle[31], Cristina Rodriguez-Fonseca[32], Rebecca Rodriguez Pena[33], Janet Russell[30], Alicia Saba[34], Fernando Santos-Simarro[35], Brittany N. Simpson[36], David F Smith[37], Markus F. Stevens[38], Katalin Szakszon[39], Emmanuelle Taupiac[1], Nadia Totaro[30], Irene Valenzuela Palafoll[22], Daniëlle C.M. Van Der Kaay[40], Michiel P. Van Wijk[41], Klea Vyshka[42], Susan Wiley[31], and Raoul C. Hennekam[10]

Author addresses:

[1] Department of Medical Genetics, University Hospital of Bordeaux, and INSERM U1211, University of Bordeaux, 33076 Bordeaux, France.

[2] Faculté de Chirurgie Dentaire, Université de Strasbourg, and Centre de référence des maladies rares orales et dentaires, Hôpitaux Universitaires de Strasbourg Strasbourg, and Institut de Génétique et de Biologie Moléculaire et Cellulaire, INSERM U1258, Illkirch, France.

[3] Department of Clinical Medicine, University of Bergen, 5020 Bergen, Norway.

[4] Department of Anesthesiology, Cincinnati Children's Hospital, University of Cincinnati College of Medicine, Cincinnati, USA.

[5] Department of Medical Genetics, Haukeland University Hospital, Bergen, Norway and Division of Evolution, Infection and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK.

[6] Institute of Medical and Molecular Genetics, La Paz University Hospital, Madrid, Spain.

[7] Department of Medical Genetics, Koc University School of Medicine (KUSOM), 34010 Istanbul, Turkey.

[8] Experimental Research Laboratory of Medical Cytogenetics and Molecular Genetics, IRCCS Istituto Auxologico Italiano, Milan, Italy.

[9] Medical Genetics Section, Department of Pediatrics, Virgen de la Arrixaca University Hospital, IMIB, CIBERER, Murcia, Spain.

[10] Department of Pediatrics, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
[11] Fondazione IRCCS Ca'Granda Ospedale Maggiore, 20122 Milan, Italy.
[12] Fondazione Matilde Tettamanti Menotti De Marchi Onlus, Fondazione Monza e Brianza per il Bambino e la sua Mamma, Monza, Italy.
[13] Department of Pediatrics, University of Tennessee College of Medicine, Chattanooga, Tennessee, USA..
[14] Groote Schuur Hospital, Department of Pediatrics, University of Cape Town, Cape Town, South Africa.
[15] Department of Pediatric Urology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
[16] Bartiméus Diagnostic Center for complex visual disorders, Zeist and Department of Ophthalmology, University Medical Center Utrecht, Utrecht, The Netherlands.
[17] School of Psychology, College of Health and Life Sciences, Aston University, Birmingham, B4 7ET UK.
[18] Université de Paris, Laboratoire de Psychopathologie et Processus de Santé, Boulogne Billancourt, France.
[19] MVZ - Humangenetik, University Medical Center, Johannes Gutenberg University Mainz, 55131 Mainz, Germany.
[20] Département de Genetique, SIDVA 91, Juvisy-sur-Orge, France.
[21] Department of Pediatric Nephrology, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
[22] Department of Clinical and Molecular Genetics, University Vall d'Hebron, Hospital Campus, Barcelona, Spain.
[23] Department of Clinical and Molecular Genetics, Fundación Rioja Salud, La Rioja, Spain.
[24] Department of Human Genetics, Amsterdam UMC, Amsterdam, The Netherlands.
[25] Sleep Education and Research Laboratory, UCL Institute of Education, London, UK..
[26] Zodiak, Prinsenstichting, Purmerend, The Netherlands.
[27] Rubinstein-Taybi Syndrome Support Group, Registered Office, Rickmansworth, WD3 3ED UK.
[28] Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.
[29] School of Psychology, University of Birmingham, Edgbaston, B15 2TT, UK.
[30] Associazione Rubinstein-Taybi Syndrome-Una Vita Speciale, Organizzazione di Volontariato (ODV), Gornate Olona, Varese, Italy.

[31] Division of Developmental and Behavioral Pediatrics, Cincinnati Children's Hospital Medical Center, and Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, OH, USA.
[32] Asociación Española para el Sindrome de Rubinstein-Taybi (AESRT), Madrid, Spain.
[33] Department of Clinical Immunology, La Paz University Hospital, and Lymphocyte Pathophysiology in Immunodeficiencies Group, La Paz Institute of Biomedical Research, Madrid, Spain.
[34] French RTS Support Group, Paris, France.
[35] Unit of Molecular Diagnostics and Clinical Genetics, Hospital Universitari Son Espases, Health Research Institute of the Balearic Islands (IdISBa), 07120 Palma, Spain.
[36] Division of Human Genetics, Cincinnati Children's Hospital Medical Center, and Department of Pediatrics, Cincinnati School of Medicine, Cincinnati, Ohio, USA.
[37] Department of Pediatric Otolaryngology, Cincinnati Children's Hospital Medical Center, and Department of Otolaryngology – Head and Neck Surgery, University of Cincinnati College of Medicine, Cincinnati, OH, USA.
[38] Department of Anesthesiology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
[39] Faculty of Medicine, Department of Pediatrics, University of Debrecen, Debrecen, Hungary.
[40] Division of Pediatric Endocrinology, Department of Pediatrics, Erasmus University Medical Centre, Sophia Children's Hospital, Rotterdam, The Netherlands.
[41] Department of Pediatric Gastroenterology, Emma Children's Hospital location Free University Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands.
[42] European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability (ERN-ITHACA), Robert Debré University Hospital, Paris, France.

Correspondence to Didier Lacombe, Department of Medical Genetics, CHU Bordeaux, INSERM U1211, University of Bordeaux, Bordeaux, France. +33 556 795951
didier.lacombe@chu-bordeaux.fr

# These authors should be considered as being second author

**Abstract**

**Rubinstein-Taybi syndrome (RTS) is an archetypical genetic syndrome that is characterized by intellectual disability, well-defined facial features, distal limb anomalies and atypical growth, among numerous other signs and symptoms. It is caused by variants in either of two genes (*CREBBP*, *EP300*) which encode for the proteins CBP and p300, which both have a function in transcription regulation and histone acetylation. As a group of international experts and national support groups dedicated to the syndrome, we realized that marked heterogeneity currently exists in clinical and molecular diagnostic approaches and care practices in various parts in the world. Here, we outline a series of recommendations that document the consensus of a group of international experts on clinical diagnostic criteria for types of RTS (RTS1: *CREBBP*; RTS2: *EP300*), molecular investigations, long-term management of various particular physical and behavioural issues, and care planning. The recommendations as presented here will need to be evaluated for improvements to allow for continued optimization of diagnostics and care.**

## 1.Introduction

Rubinstein-Taybi syndrome (RTS) [MIM #180849; #613684; #610543] is a multisystem disorder with physical, cognitive and behavioural characteristics, which can be caused by variants in two genes that regulate transcription via chromatin remodelling. The condition is named after the US paediatrician Jack Rubinstein and Iranian radiologist Hooshang Taybi who described seven affected infants in 1963,[1]. There are >800 publications on RTS and related topics. Within the framework of the European Reference Network Ithaca a group of international experts recognised the importance of equal practices regarding diagnostic procedures and care for individuals with RTS. To address this issue, an international consensus group was established, which performed a literature review, evaluated data critically, formulated conclusions, and held a face-to-face meeting in the presence of patient group representatives. This has led to the present series of guidelines for diagnostics and care for individuals with RTS. For Methods see Supplementary Materials.

## 2. Clinical Diagnostic Criteria

### *2.1. Definition*

The goal of defining an entity is that affected individuals and their caregivers who face similar signs, symptoms, and health problems, can meet one another, share knowledge, emotions and experiences about the disorder, support one another, and, this way, facilitate care and research. So the essence of a definition is to allow grouping together individuals with the same diagnosis.

Currently, variants in the genes *CREBBP* and *EP300* are known to cause RTS,[2,3]. One may argue that the diagnosis of RTS should be based on these molecular findings and clinical diagnostic criteria are no longer needed. Several issues argue against this: there are individuals with a phenotype classically fitting RTS, but without detectable cytogenetic or molecular anomaly; there are individuals with a genuine variant in *CREBBP* or *EP300* but with a phenotype different from the RTS phenotype,[4] which can have major consequences in counselling patients and families; there are individuals with either a *CREBBP* or an *EP300* variant of uncertain pathogenicity, and whose phenotype resembles RTS only to a limited extent, leaving it uncertain whether or not the variant is causative for the phenotype; there are many countries worldwide in which the availability of molecular studies is limited, and in which caregivers have to rely on a clinical diagnosis for counselling. For these reasons we concluded a clinical definition of the RTS phenotype is still needed and will remain needed.

There is no widely accepted set of clinical diagnostic criteria for RTS. We used the largest published set of data on individuals with RTS and either a *CREBBP* (n=308) or *EP300* variant (n=52),[5] to determine the sensitivity of signs and symptoms (Table 1).

We used the scored features as available, to avoid a bias. Signs present in at least 75% in either of the two groups were accepted as being sufficiently characteristic of the condition. In addition, we added three features with a lower frequency but which are highly specific for RTS: radially deviated thumbs; keloid formation; and maternal pre-eclampsia. We considered to add talon cusps to these criteria but refrained from doing so as this sign is not yet present in the age group during which typically a diagnostic question arises. When developing the scoring system, it was observed that the presence or absence of the sign 'long eyelashes' did not contribute to sensitivity, and given the low intra-observer reliability of this feature it was excluded from the scoring criteria. Furthermore, the features known to be highly specific for RTS (radially deviated thumbs typical smile; columella below alae nasi, maternal pre-eclampsia keloids) were given a higher weighted value in the scoring system to reflect their diagnostic importance. Features were then subdivided into Cardinal Features, which we considered to be essential for RTS, and Suggestive Features, which are present less frequently but should raise suspicion for RTS (Table 2; Figure 1). Subsequent discussion of these criteria allowed consensus for the clinical diagnostic criteria, based on the presence of both Cardinal and Suggestive Features (**R1**). If an individual scores 12 or higher, including meeting a score for the Cardinal Features, the diagnosis of RTS can be clinically confirmed irrespective of results of molecular testing. A score of 8-11 including a positive score for the Cardinal Features indicates a likely diagnosis of RTS which requires further confirmation by molecular testing. A score of 5-7, with or without a Cardinal Feature, indicates that the diagnosis of RTS is still possible and molecular studies are indicated. A score of 0-4 indicates that the diagnosis is unlikely, and other explanations of the phenotype should be explored.

We realize that the presence of unusual signs and symptoms is not incorporated in the score as negative feature. Still, these should always also be taken into account. Especially the presence of an unusual sign or symptom in someone with a score indicating a likely or definitive diagnosis of RTS should lead to consider the presence of a co-existing second (possibly Mendelian) disorder. In addition, in scoring signs, especially low hanging columella, the ethnic background should be taken into account as in some ethnicities a low hanging columella is a common variant. If uncertainty remains it is often useful to evaluate both parents and other relatives as well (**R2**). Lastly, in the first months of life a delayed development and disturbed postnatal growth may not yet present and a definitive score may be only possible at an age when this can be reliably ascertained.

Subsequently, we evaluated whether the set of diagnostic features allowed establishing the diagnosis reliably in a group of 100 individuals with molecularly confirmed RTS, that had not been part of the group of patients on which the criteria were built (Suppl Table S1). All individuals scored 5 or higher, indicating none would have been missed as having RTS based

on clinical criteria (complete sensitivity). Only 7 patients scored in the group Possibly RTS, others scored in the group Likely RTS (n= 38) or Definitively RTS (n=55). Furthermore, we evaluated whether 45 individuals with a specific group of pathological *CREBBP* or *EP300* variants, who have been considered to have a separate entity (Menke-Hennekam syndrome [MKHK]; MIM #618332 / #618333),[4] would be correctly distinguished from RTS (Suppl Table S1). Results showed that none scored as definitive or likely RTS, 9 as possibly RTS, and 36 as unlikely RTS, so the entities could correctly be discerned. To determine the specificity, we reasoned that three entities that may resemble RTS and are not uncommon, i.e. Floating-Harbor syndrome (FHS; MIM #136140) (n=45), Wiedemann-Steiner syndrome (WDSTS; MIM #605130) (n=46), and Cornelia de Lange syndrome (CDLS; MIM #122470) (n=100), should be reliably discerned from RTS based on the set of weighted clinical features (Suppl Table S2). Results showed that none of the individuals with FHS and CDLS fulfilled the criteria for a definitive diagnosis of RTS, but one of the WDSTS patients had such a score. In addition, one of the WDSTS patients had a score within the Likely RTS group but was found by the present authors to have a classical RTS facial Gestalt. This has to be expected as RTS is a chromatinopathy, and variants in other genes acting in the same pathway are likely having consequences for the phenotype as well and rarely may even alter the phenotype significantly. Further studies to explain this unusual phenotype are planned. Furthermore, 8 of the 46 WDSTS individuals, and 1 of the 100 CDLS individuals fulfilled the criteria for Likely RTS, indicating that specificity was very high, but not complete. Due to the overlap in function of the genes involved in the four entities this is to be expected,[6]. The results are in agreement with our joint clinical experience that infrequently the discrimination between RTS and WDSTS based on clinical criteria can be extremely difficult. This happens less frequent in CDLS patients and in FHS, but the phenotypic overlap is still marked. Obviously, this has consequences for the molecular analyses in someone with such scores (see *Molecular Diagnostic Criteria*). We realize that prospective studies will be needed to determine more reliably specificity and sensitivity. In addition, such studies should include individuals with a non-European descent, to evaluate whether the scoring system will be equally valid as in individuals from a European descent.

### *2.2. Severity Score*

A major issue for families, especially at the time of diagnosis, is an indication of the severity of RTS. No severity score for RTS has been published to date. In our opinion a comparison and weighing of the severity and influences that various signs and symptoms have on the quality of life of an affected individual can only be made by the affected individuals and their families, and not just by physicians. We suggest that a group of family members should be facilitated to indicate which set of physical, cognitive and behavioural issues influence the life of individuals

with RTS most. Ideally, such criteria should be stratified according to the nature of the molecular genetic cause (**R3**).

## 3. Molecular diagnostic criteria

RTS has been subdivided into type 1 (RTS1; OMIM #180849) and type 2 (RTS2; OMIM #613684) associated with heterozygous pathogenic variants or re-arrangements in the genes *CREBBP* and *EP300*, respectively, typically leading to haploinsufficiency. Both genes encode paralogous transcriptional coactivators with Lysine Acetyl Transferase Activity,[7,8]. The proteins CBP and p300 play a crucial role in transcription initiation by acting as a bridge, linking transcription factors to the transcription machinery, and through acetylation of histones,[9,10] (Figure 2).

### *3.1. Mutation spectrum*

Variants in *CREBBP* and *EP300* have been identified in 55-75%,[2,3,11,12] and 8-11%,[3,5,13,14] of individuals with RTS, respectively, of whom 2-3% have deletions of the complete gene. In 15-20% no molecular anomaly can be detected (**R4**). To date, over 500 *CREBBP* and over 100 *EP300* pathogenic variants are known, distributed along all 31 exons (Figure 3). Several recurrent *CREBBP* variants have been reported, ~50% of missense variants are localized in the KAT domain,[15] and recurrent rearrangements occur between introns 1 and 2 of *CREBBP* due to the high frequency of repeated or palindromic sequences in this region,[16,17].

### *3.2. Genotype-phenotype correlation*

Individuals with RTS1 and RTS2 both may show the classical phenotype but this may also vary. Individuals with RTS2 demonstrate in general less marked typical facial characteristics, no radial deviation of the thumbs, have infrequently keloids, and a higher average cognitive level,[5,13,14]. However, maternal pre-eclampsia, intra-uterine growth retardation and microcephaly are more common in RTS2 compared to RTS1,[5].

The type and site of variants in *CREBBP* and *EP300* do not associate with a specific phenotype with respect to external morphology, malformations, cognition or behavior,[5,11,13,18,19] (**R5**). The exception is formed by missense variants between the end of exon 30 and the beginning of exon 31 of both *CREBBP* and *EP300*, which both lead to a phenotype that differs from RTS (Table 1) and has been designated as Menke-Hennekam syndrome (MKHK, OMIM #618332, #618333),[4,20]. These missense variants hypothesized to affect specifically the binding properties of the ZNF2 (zinc finger, ZZ type) and ZNF3 (zinc finger, TAZ type) domains to different CBP partners by affecting their own folding,[21,22].

RTS shows broad phenotypic overlap with other Mendelian disorders affecting the structure of chromatin genome-wide called “chromatinopathies”, such as Floating Harbor syndrome (OMIM #136140), Cornelia de Lange syndrome (OMIM #122470, #300590, #610759, #614701, #300882, #608749), Wiedemann-Steiner syndrome (OMIM #605130), Kabuki syndrome (OMIM #147920, #300867), Genitopatellar syndrome (OMIM #606170), Biesecker-Young-Simpson syndrome (OMIM #603736) and Gabriele-De Vries syndrome (OMIM #617557).

### *3.3. Diagnostic approach*

There are two main entry points for molecular genetic testing in RTS: clinical suspicion of RTS or no clinical suspicion (Figure 4). If clinical presentation suggests RTS, the first-line tests are either targeted analysis of *CREBBP* and *EP300* by Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) or by high throughput analysis (array Comparative Genomic Hybridization [aCGH]; Whole Exome Sequencing [WES] if accessible). If RTS is not suspected in an individual with intellectual disability and/or malformations, the first tier is high throughput analyses (aCGH; WES or Whole Genome Sequencing [WGS]). Evaluation of variant should be performed using the ACMG classification,[23]. Additional RNA studies are needed in case of unknown splicing variants. Suspicion of somatic mosaicism should be confirmed in more than a single tissue (buccal swab; bladder epithelium cells; skin biopsy). The phenotype should be re-evaluated after identification of a (possibly) pathogenic variant to confirm that the molecular finding fits the clinical phenotype. If targeted analyses yield negative results and high throughput analyses are not available, the diagnosis remains dependent of the clinical phenotype and a definitive diagnosis may not be possible.

If the clinical diagnosis cannot be confirmed molecularly, molecular analyses yield a variant of unknown significance (VUS), or the phenotype does not fit the molecular finding, analysis of a genome-wide methylation pattern (epigenetic signature) can be performed as individuals with RTS have a specific pattern,[24].

If all studies are negative, one should consider other diagnoses. Still, currently not all molecular mechanisms leading to RTS are known, and if the clinical diagnostic criteria for RTS are met (see *Clinical Diagnostic Criteria*), the diagnosis RTS remains the standard in guiding management and follow-up of the patient.

### *3.4. Recurrence risk*

RTS is inherited as an autosomal dominant trait and occurs *de novo* in over 99% of patients. However, familial occurrence does occur, either if a parent is relatively mildly affected or due to somatic or germ-line mosaicism,[25,26]. To date, eight instances of somatic or germ-line mosaicism and seven instances of parent-to-child transmission have been described in over

2000 reported affected individuals, indicating the empirical recurrence risk is 0.5-1%,[27]. The recurrence risk for offspring of an affected individual is 50%, although it may be lower due to a spontaneous miscarriage (**R6**).

*3.5. Prenatal diagnosis*

Without a positive family history, the prenatal diagnosis of RTS is only infrequently made as there are few reliable antenatal signs. Truly detailed three-dimensional ultrasonography may allow suggestive facial characteristics, but the morphology of the extremities, and specifically the radially deviated thumbs, are the main diagnostic handles,[28,29]. Additional findings that may be helpful are intra-uterine growth retardation, polyhydramnios, underdevelopment of the cerebellum, and gallbladder anomalies,[26].

The main reason to perform prenatal diagnostics for RTS is the birth of a previous child with RTS in the family. If a causative variant in *CREBBP* or *EP300* has been detected, reliable molecular prenatal diagnostics can be performed in samples obtained by chorionic villus sampling or amniocentesis, or in embryonic cells obtained by *in vitro* fertilization (**R7**).

Prenatal testing in families without a previous child with RTS and known pathogenic variant, by non-invasive cell-free fetal DNA screening, is not advocated, as interpretation of pathogenicity of variants detected this way may be extremely difficult. This limits validity and informative value of the prenatal testing and may cause ethical issues for the families in deciding whether or not a pregnancy should be continued. Any prenatal testing needs be discussed carefully with the couple before the procedure and should take into account the differences in perspective of couples and national legislation.

## 4. Neonatal care

*4.1. Recognition*

86% of children present within the first month of life and 70% of these on the first day of life; prolonged hospital admission after birth was reported in 61%,[30]. Early recognition of RTS may help identify complications and assist families to cope,[31]. The typical facial features of RTS evolve with time,[32]. The characteristic appearance in the neonatal period differs somewhat as it is mainly characterized by a prominent forehead with haemangiomas (‘stork-bite naevus’) in the glabella region, (apparent) hypertelorism, epicanthi, and at that age up-slanting palpebral fissures. The nasal bridge tends to be straight, the tip short and upturned, and the nasal septum is not or only slightly extending beyond the alae,[32]. A small mouth, highly arched palate, and small mandible are also present. Additional features can be unusual thick, black hair, a large anterior fontanelle, and long eyelashes. Newborns with a variant in

*EP300* tend to have a less obvious phenotype,[5]. The distal limb anomalies are the most characteristic for RTS in the neonatal period and are similar to those at an older age (see *Clinical Diagnostic Criteria*). Cryptorchidism is common.

*4.2. Feeding*.

Neonatal feeding difficulties are common (71-80%), due to swallowing incoordination, poor nipple grasp, hypotonia and gastro-oesophageal reflux,[33]. Nutritional supplementation including gastric tube feeding is required in 40% of cases, as are occasionally percutaneous tubes, but most feeding challenges will have resolved within the first year of life,[30]. Should feeding difficulties persist, additional professionals should be consulted (see *Gastroenterology*). Still, half of the mothers report a sufficient suck and were pleased with their breastfeeding experience,[33]. Adequate breastfeeding instructions, proper positioning, and ongoing encouragement are indicated (**R8**).

*4.3. Birth parameters*

At birth most infants fall within the normal range for weight, length and head circumference,[34] although a higher incidence of microcephaly and growth restriction has been reported in infants with *EP300* variants, possibly related to the frequently occurring pre-eclampsia,[5]. There is no increased risk of preterm birth,[35]. The use of RTS specific growth charts is encouraged to monitor growth adequately (**R9**).

*4.4. Systemic manifestations*

The various systemic manifestations of RTS are described elsewhere in the guidelines. The work-up of every newborn with suspected or confirmed RTS should include ophthalmological exams (glaucoma; coloboma); cardiac assessment (malformations); and renal ultrasound (malformations)(**R10**). Obviously, further care such as the baseline new-born hearing screening and vaccinations should be performed as per the general population.

## 5. Endocrinology

*5.1. Hypoglycemia*

Transient hypoglycemia occurs with a low frequency in newborns with RTS and responds well to usual management schemes (**R11**). Hypoglycemic hyperinsulinism (HH) is very rare, may occur after birth or in the first years of life, sometimes associated with concurrent illness, and can be transient or permanent,[36,37]. It has mainly been described in children with *EP300* variants,[5]. Early diagnosis and treatment of HH is crucial to avoid permanent brain

damage,[38]. Treatment is as in the general population: frequent enteral feeding, continuous glucose infusion, diazoxide). Usually specialist consultation is needed,[39].

*5.2. Growth*

Postnatal growth retardation is a hallmark of RTS,[34]. Usually within months after birth, the length, weight, and head circumference drop from normal values to ~ -2SDS. Neither boys nor girls show a pubertal growth spurt, which contributes to a subsequent average adult height of -3SDS for both males and females,[34]. The use of growth charts specific for RTS, based on molecularly confirmed patients, facilitates adequate monitoring of growth (**R9**). Growth hormone (GH) deficiency is infrequent but has been reported in few individuals, in whom GH therapy resulted in an increase in height SDS,[40]. Every child in whom growth differs markedly from the growth pattern of the dedicated growth charts, needs to be evaluated for GH deficiency (**R12**). If present, treatment is as in the general population. Pre-pubertal boys and girls may develop an unusual body shape due to increased fat tissue around abdomen and hips, which disappears in puberty in boys, but often persists throughout life in girls,[41].

*5.3. Puberty*

The timing of puberty and development of secondary sex characteristics usually falls within normal limits. Mean age of onset of puberty was 12.2 years,[35] with mean age of menarche at 13.6 years,[41]. There is no indication fertility is decreased, although formal studies are lacking. About 25% of adult males and females with RTS are sexually active,[42]. Sexual education should be proposed according to the level of emotional and cognitive functioning,[43] and contraceptive options are recommended as in the general population taking the level of developmental functioning into account (**R13**).

## 6. Gastroenterology

Malformations of the gastro-intestinal tract such as a duodenal web and malrotation occur at a low frequency in newborns with RTS, although the frequency of the malrotation may be higher than in the general population,[44,45]. Symptomatology is similar as in newborns without RTS and should be managed as in the general population,[41,45].

Feeding problems are very frequently present at birth and may remain present for a prolonged period of time,[41,45,46]. Oral feeding is preferred if it is safe and feasible, while tube feeding may be needed and a gastrostomy for long-term use. Involvement of dieticians is often helpful (**R14**). Although feeding problems are in part explained by the recurrent respiratory infections and hypotonia, also gastro-oesophageal reflux (GOR) may play a role,[46]. Limited GOR occurs in all healthy infants and children; if causing excessive

symptoms it is referred to as GOR disease (GORD),[47]. The symptomatology of GORD may vary widely, from feeding problems, dental enamel erosions, and recurrent pneumonias to restlessness and poor sleep. The pathogenesis remains uncertain,[46]. GOR(D) should be differentiated from excessive regurgitation after feeds in otherwise asymptomatic infants, which is usually indicated as infant rumination syndrome,[48]. Extremely rarely, eosinophilic esophagitis may develop,[49]. Given the lack of evidence for management of GORD specifically in RTS, management of GORD should be as in the general population,[47]: thickening of food and reassurance of parents as a first step. If symptoms persist, an initial trial with PPI treatment can be considered. If problems continue, further evaluation should be considered. If a PPI trial improves symptomatology, this does not conclusively prove acid-related GORD. Long term use of PPI may cause side-effects,[50] thus in successful PPI trials individuals should undergo weaning trials regularly (e.g. after 6 months and yearly thereafter) to evaluate the utility of continuing PPI treatment, while mitigating rebound effects by dose tapering. If symptoms persist or recur, additional testing, such as pH-impedance testing and/or endoscopy can be considered (**R15**). Fundoplication and other surgical interventions are not recommended in an early phase of management, as these have a relatively high failure rate, commonly cause complications, and can induce dysphagia and subsequent feeding problems; it should be reserved for patients with proven GORD unresponsive to optimal nutritional and medical therapy,[51]. Fortunately, complications of long-term GORD such as Barrett oesophagus are rare in RTS,[52] and oesophageal cancer has not been reported.

Constipation is extremely prevalent in RTS across all age groups throughout the lifespan,[41,45]. The cause remains unknown, Hirschsprung disease or other identifiable etiologies do not occur more frequently than in the general population. Additional investigations are only indicated if symptomatology suggests an underlying disease. Long-term treatment with increased dietary fibers and fluid intake, and oral osmotic laxatives remain the cornerstone of treatment,[53] **(R16)**. In severe cases, stimulant laxatives may be added, and further management schemes are as in the general population.

## 7. Cardiology and Pulmonology

### *7.1. Cardiovascular system*

Congenital heart defects (CHDs) occur in 30% of cases, without a genotype-phenotype correlation,[18,54–56]. The reported differences in incidence according to ethnicity can be explained by ascertainment bias and differences in methodology,[57]. The typical CHDs are patent ductus arteriosus, persistent foramen ovale, and atrial and ventricular septal defect,[5,13,19,55,58–60]. Individuals with a CHD do not have a higher rate of other malformations or are associated with impaired cognitive function.

The cardiovascular system should be evaluated at diagnosis, including cardiac sonography (**R17**). Treatment is as in the general population, including endocarditis prophylaxis as indicated. Surgery is needed in 15-22% of patients,[42,61]. CHDs do not cause unexpected complications in adults,[42].

Cardiovascular problems typical for the general adult population occur in adults with RTS in a lower frequency. Hypertension is reported in 10% of adults,[42] and surveillance and treatment are as in the general population (**R18**).

### *7.2. Pulmonary system*

Mild respiratory distress in the first hours of life is common in RTS neonates. Treatment is only needed if other risk factors such as prematurity are present. Upper respiratory infections are common (see *Immunology*). Infections of the lower respiratory system are uncommon,[42] and are explained by feeding problems, micro-aspirations, and gastro-oesophageal reflux. Exceptionally, an immunodeficiency may play a role; the reported higher frequency of lower respiratory infections was caused by a study bias,[62]. In case of recurrent pneumonia with wheezing, hoarseness, or stridor, the patient should first be evaluated for micro-aspirations and gastro-oesophageal reflux,[49] (**R19**). If negative, a search for immunodeficiency is indicated. Bronchiectasis has been described only in individuals with severe immunological malfunctioning,[63].

Interstitial lung disease that becomes evident either in childhood,[64] or adulthood,[65] is uncommon but potentially severe. The diagnosis is made through the radiological characteristics on computed tomography and can be confirmed by biopsy,[64]. Management is as in the general population and is problematic.

Pulmonary functioning can also be compromised secondary to restrictive pulmonary diseases related to scoliosis,[66] and pulmonary hypertension caused by chronic sleep apnoea (OSA),[67] (see *Ear Nose and Throat*).

## 8. Ophthalmology

Ocular abnormalities and/or reduced vision are reported in 20-80% of individuals with RTS,[55,57,61,68–72]. An overview of ocular anomalies is presented in Table S3 (Suppl Materials). Every child with RTS needs to be referred for ophthalmological evaluation once the diagnosis is suspected (**R20**).

Eye abnormalities were reported to be more common in individuals from Asia and Latin America than those from Africa and the Middle East, but this may be biased,[57]. Both individuals with *CREBBP* and *EP300* variants present ocular anomalies, but due to small

numbers of data on individuals with *EP300* variants differences in occurrence remain uncertain.

*8.1. Anatomical anomalies*

Congenital nasolacrimal duct obstruction by a persistent membranous obstruction at the entrance of the duct into the nose causes a watery eye from birth. It is mostly unilateral, with the incidence between 11% – 47%,[55,57,59,71–74]. Treatment follows international guidelines (Nasolacrimal Duct Obstruction in Children - American Academy of Ophthalmology (aao.org)) but the surgeon should be aware of the thicker bones and brittle lacrimal sacs in children with RTS,[75].

The reported frequency of congenital glaucoma varies from 4%-11%,[55,57,61,72,75]. The glaucoma can be unilateral or bilateral and be associated with anterior segment anomalies such as iris coloboma or lens luxation. Symptoms include tearing, blepharospasm, and photophobia, and enlargement of the eye, manifesting as megalocornea and rapidly increasing myopia. Treatment should be as soon as possible after birth as it can lead to marked loss of vision (www.eugs.org. Congenital Glaucoma - Europe - American Academy of Ophthalmology (aao.org)).

Cataract has been reported in 6-25% of individuals with RTS,[19,57,61,72,75], and is usually congenital,[72]. Reliable incidence figures are lacking. Early diagnosis and treatment in the first two months of life are mandatory to avoid visual deprivation, treatment is as in the general population (Pediatric Cataracts: Overview - American Academy of Ophthalmology (aao.org). Frequent follow-up is needed for appropriate refractive correction and monitoring of secondary complications. Cataract may also develop later in life,[71] (**R21**).

Coloboma is reported in 10% of individuals,[19,57,59,71–73]. The coloboma can affect the iris, choroid, retina, and/or optic nerve. Symptoms depend on location and size and may include visual field loss, reduced vison and photophobia. There is no curative therapy, but sometimes glare can be reduced by wearing sunglasses.

Retinal abnormalities occur frequently,[72] but are often subtle, so may go unnoticed, without severe loss of vision, except for macular degeneration secondary to high myopia (**R21**). Evidence may be present in abnormal distribution of pigment in the macula and a subnormal electroretinogram. In some patients, the abnormal aspect of the macula is caused by foveal hypoplasia (Van Genderen, unpublished).

*8.2. Functional anomalies*

Visual impairment (best corrected binocular visual acuity < 6/18) occurs in 20% of individuals,[72] and typically is caused by anatomical abnormalities. Bilateral severe anomalies may lead to infantile nystagmus because of decreased sensory input from birth. Refractive

errors and strabismus are very common, both occurring in 50-75% of individuals, and may change rapidly with age indicating the need of frequent controls, especially under 5 years of age[13,55,57,61,71,72] (**R21**). In young children, high refractive errors need correction to prevent amblyopia. Children may however refuse to wear glasses if improvement of vision is not immediately evident. Gradual introduction in situations in which the child benefits most from glasses may allow the child to get accustomed to wearing spectacles (**R22**).

Treatment of strabismus to prevent amblyopia is as in the general population, provided the affected eye has no congenital anomaly that inhibits amelioration of vision.

Photophobia is common due to cataract, glaucoma, or trichiasis,[72] treatment is by treating the cause. Photophobia secondary to coloboma or retinal dysfunction can be ameliorated by shielding the eyes from direct (sun) light or wearing sunglasses.

## 9. Otolaryngology and Anesthesiology

### *9.1. Hearing*

The typical facial characteristics in individuals with RTS include a small chin and small oral cavity which can result in airway difficulties and, together with gastro-oesophageal reflux, can result in complications as recurrent middle ear infections,[76]. Conductive, sensorineural and mixed hearing loss may result,[77–79]. Regular auditory evaluation is therefore recommended **(R23)**.

### *9.2. Sleep*

Abnormal facial anatomy and increased collapsibility of the laryngeal walls predispose individuals with RTS to higher rates of sleep disordered breathing and obstructive sleep apnea,[80,81]. Sleep disorders are frequent in children, and occur in 62% of adults,[42,61]. Obstructive sleep apnea (OSA) is typically characterized by snoring and excessive daytime sleepiness, and affects 25% of adults with RTS,[42,61]. If present in children the facial anatomy is often markedly abnormal and accompanied by obesity, hypotonia and adeno-tonsillar hypertrophy,[81]. As with the general population, management should take into account the various causal factors as well as potential difficulties in treating both children and adults with RTS,[67] (**R24**). Assessment of the sleep patterns using a validated questionnaire, such as the Sleep Disturbance Scale for Children,[82] may offer information on both sleep patterns and response to therapy (**R25**). Prior to a major surgical intervention, polysomnography should be considered,[83]. Management of sleep disorders is aimed at implementing healthy sleep practices, particularly position during sleep, behavioral strategies, and the use of and

education on pharmacologic interventions. Melatonin should be used appropriately in individuals with specific types of insomnias and sleep rhythm disturbances.

*9.3. Anesthesiology*

Approximately 48% of adults with RTS require surgery at least once, with half of those requiring two or more surgeries during their lifetime,[42]. Children with RTS are no exception as they receive a higher fraction of anesthetics relative to their age-matched cohorts,[35]. As a result of the multi-systemic manifestations of RTS, anesthesiologists should be prepared to provide a tailored anesthetic for this population **(R26)**.

Premedication and behavioral therapy support may prove beneficial in the preoperative setting. A single case series described complications such as cardiac arrhythmias associated with intraoperative administration of atropine and succinylcholine, but other studies have shown the safe and efficacious use in RTS,[84,85] and this is also our joint personal experience. The altered facial anatomy may make mask-ventilation, laryngoscopy, and intubation challenging, and coupled with positioning limitations that may be present due to scoliosis, kyphosis, hypermobility, and obesity, may warrant use of video-laryngoscopy or fiberoptic intubation,[35,86]. Rarely, transnasal placement of a nasopharyngeal airway or nasogastric tube is inhibited due to narrow or atretic choanae.

Intraoperative management of ventilation and post-extubation care can be complicated by the presence of laryngotrachomalacia and augmented airway reactivity. In the immediate postoperative period, opioid use, while not contraindicated, should be used judiciously to prevent exacerbation of obstructive symptoms and hasten potential apneas. The peri-operative use of analgesic and anxiolytic adjuncts such as NSAIDs, acetaminophen, and dexmedetomidine are encouraged, if not contraindicated secondary to other co-morbidities or surgical considerations. Initiation of transient, non-invasive positive airway pressure may be helpful. Secondary to the elevated risk of complications with anesthesia and airway manipulation, particular efforts should be made to bundle non-emergent procedures into a single anesthetic to mitigate potential morbidity (**R27**).

## **10**. **Dermatology**

The main skin problem in RTS is the propensity to develop keloid. Keloids are non-malignant fibrous growths resulting from an abnormal response to skin injuries or inflammation that extend beyond the borders of the original wound. The pathogenesis of keloids is thought to involve multiple patient-specific factors (genetics, age, hormones, ethnicity), and environmental factors (trauma, surgery, inflammation) which collectively stimulate wound healing and persistent inflammation,[87]. Spontaneous keloids occur only in genetic

syndromes,[88] raising the question whether they are truly spontaneous, or whether unrecognized triggering environmental factors occur.

RTS is the syndrome considered to have the highest risk of keloid development,[89]. The frequency of Dutch and UK RTS individuals developing keloids was 24%[89]. While keloids are most frequently occurring in association with *CREBBP* variants, around 10% of individuals with *EP300*-related disease develop such changes,[5,13,56]. Compared to the general population keloids develop earlier in life in individuals with RTS,[57,89] and increase with age: up to 60% was reported in a cohort of adults,[42]. Up to 100 keloids have been recorded in the same individual,[90]. In RTS keloids are most frequently seen on shoulders and chest,[89]. Development of keloids is not associated with other traits of the phenotype within RTS,[89].

Apart from aesthetic issues, keloids cause pain, itching and reduced mobility of the involved region, thus seriously affecting the quality of patients' lives,[89] (**R28**). Prevention is difficult and keloids may be unavoidable as minimal trauma such as rubbing of clothes may be sufficient to induce keloid formation. There are no standardized treatment protocols of keloids in individuals with RTS. Therapy options include repeated intra-lesion steroid injections, laser therapy, compression, local radiation, cryotherapy, and surgery, either individually or in combination, but no treatment is fully satisfactory, and the recurrence rate remains high,[91]. There is no detectable association between keloids and cancer risk, suggesting different etiologies or pathogeneses,[92].

Another skin problem in RTS occurring in 17% of a series of molecularly proven Dutch cases,[93] are multiple pilomatricomas: benign skin tumors derived from hair matrix, often harboring activating mutations of beta-catenin,[94]. These skin-colored, red, or white lesions typically occur on the head and neck in children and adolescents, but do occur elsewhere and may arise at older ages as well. Pilomatricomas typically calcify, causing them to feel like hard lumps. They may coexist with keloids,[19,95]. Similar to keloids there are often multiple pilomatricomas, and puberty may act as triggering factor. Complete surgical excision has been recommended,[96], but others suggested surgical removal only in case of discomfort,[89] (**R29**).

Ingrowing nails occur regularly in both fingers and toes, especially in the partially duplicated thumbs and halluces,[35] and may cause pain and skin infections. Adequate instructions regarding nail care and avoiding narrow shoes may prevent ingrowing nails (**R30**). Treatment is as in the general population. Further skin findings in RTS are congenital generalized hypertrichosis, both in individuals with *CREBBP* and *EP300* variants,[97] apparently more frequent in individuals from Latin America and Middle East and less frequently in those from Africa,[57]. Usually, it becomes less marked with age. Other changes are

angiomas, melanocytic naevi, white papulae on trunk and limbs, supernumerary nipples, and sometimes lentigines and café-au-lait spots.

## 11. Urogenital system

### *11.1. Urinary Tract*

Urinary tract anomalies occur in 23% of individuals with RTS,[5,13,19,35,55,57] and include horseshoe kidney, renal duplication, renal agenesis, renal dysplasia, hydronephrosis, nephrolithiasis, and vesicoureteral reflux. Symptomatology and treatment follow the general population management. Individuals with *CREBBP* and *EP300* variants are equally affected and there is no known genotype-phenotype correlation.

The high prevalence of renal anomalies warrants at least one renal ultrasound and blood pressure measurement when the diagnosis of RTS has been made (**R31**). If renal anomalies or an elevated blood pressure are detected, consultation with a specialist ([pediatric] nephrologist and urologist) is recommended (**R32**). Hypertension in children with RTS is rare but can occur, and is then caused by renal artery stenosis (RCH, unpublished observations).

### *11.2. Genitalia*

The most common genital anomaly is unilateral or bilateral cryptorchidism, which occurs in 59% of males,[13,17,19,35,55,57]. All males should be checked by careful physical exam after diagnosis (**R33**). Treatment is as in the general population following international guidelines,[98]. Other external anomalies occurring in less than 10% of individuals are hypospadias in both males and females, and fusion of labia minora,[19,35] which can be treated as in the general population. Shawl scrotum formation is common in RTS and needs no treatment.

Uterine malformations have been reported rarely,[99]. Females may have hypermenorrhagia or metrorrhagia. A questionnaire survey among 76 females (Suppl Materials Menses Survey) yielded that 10 of them did not yet or did no longer menstruate, 21 of the remaining 66 (32%) used medication (typically contraceptives) because of menses problems, 19 of the 45 (42%) without this medication has metrorrhagia and 10 of 45 (22%) menorrhagia. Contraceptives were invariably successfully treating the menses problems (**R34**).

## 12. Musculoskeletal System

Musculoskeletal anomalies in RTS vary widely. They are somewhat more frequent in individuals with *CREBBP* variants than in those with *EP300* variants,[5]. Using the data from

several large series of patients,[5,11,13,17–19,100–103] major limb anomalies (*CREBBP* variants vs *EP300* variants) are broad thumbs (343/360; 95% vs 51/81; 63%), radially deviated thumbs (183/343; 53% vs 5/71; 7%) and broad halluces (278/290; 96% vs 55/81; 68%). The broadness of the thumbs hardly ever causes problems, but the broadness of the halluces may cause problems in walking or wearing shoes, especially if the halluces are medially deviated. In a minority of patients, surgical correction is needed. Several methods for surgical correction have been reported,[104–107]. However, often the deviated thumbs have good function and recurrence of the deviation after surgery is common. In our experience a decision regarding surgery is best postponed until the function of the hands in the patient can be accurately evaluated, which typically can be done around 3 to 4 years of age. If surgery is indicated, it should be performed by a surgeon familiar with the procedure in RTS (**R35**).

Other findings include limitation of mobility between the proximal and distal phalanx of the thumbs, broadness of distal phalanges of fingers, limited syndactylies, and rarely camptodactyly, but these do not require treatment.

Hypermobility in the hip, elbow, fingers and thumbs, knee and patella is common,[35,80,108,109]. In combination with other not well-known factors (muscular, bony, neurologic), this may cause stiffness and the typical waddling gait in some adolescents and adults. A detailed evaluation of motor skills is indicated,[110] (**R36**). Further studies describing gait problems in RTS are lacking.

Regular evaluation of the gait is indicated since patella dislocation and Perthes-like hip problems may need therapy (**R36**). In particular, patella problems can cause major mobility challenges and, if untreated, can cause problems like genua valga and knee contractures. These issues may ultimately necessitate wheelchair use. Recurrent patella dislocation may require physical therapy, orthotics or surgical correction,[111,112] although procedures are not always successful.

An emerging gait disturbance in older children and adolescents may be caused by an aseptic hip joint inflammation resembling Perthes disease, which occurs in 3% of patients, is often marked, and may take 2 or 3 years to resolve spontaneously,[80]. It may be difficult to distinguish this from slipped capital femoral epiphyses,[113]. Management is symptomatic.

Other uncommon limb problems such as congenital hip dislocation, tight heel cords and increased risk for fractures, should be treated as in the general population.

Scoliosis is reported in 34/184 (18%) of individuals with *CREBBP* variants and 15/78 (19%) of those with *EP300* variants,[5] and develops in late childhood and puberty. Treatment is as in the general population (**R37**). Significant thoracic kyphosis and lumbar lordosis can occur and typically do not need treatment,[41,45]. Radiologically the spine may show changes resembling an early ankylosing spondylitis (M. Bechterew) but progression into a true ankylosing spondylitis has not been reported,[35]. Other infrequent spine anomalies include

instability of C1-C2, underdevelopment of the dens, and cervical vertebral fusions, which should be managed as in the general population,[114]. Occult spina bifida is detected regularly but does not cause clinical manifestations and may be left untreated.

Children and adults have an increased fracture risk, and 8% of adults have osteoporosis indicating a potentially disturbed ossification in RTS,[42] [Simpson *et al.* unpublished observations] (**R38**). Clues for this abnormal ossification in radiographies of the upper spine have been reported,[35].

## 13. Intra-oral characteristics

The main non-dental oral characteristic of RTS is the narrow, highly arched palate, that may rarely show clefting of either the complete palate (sometimes submucous), the soft palate or only the uvula, which may or may not be accompanied by a cleft lip,[5]. A careful evaluation of the palate is indicated in every newborn or child with RTS (**R39**). The treatment of clefting is as in the general population. Other, less frequent characteristics are a relatively large tongue, bifid tip of the tongue, a short frenulum, and wide alveolar ridges,[35].

Dental characteristics are almost universally present and may exist as abnormalities in tooth number (15-30%; hyperdontia, hypodontia, mesiodens), structure (23-29%; enamel hypoplasia, discoloration), eruption (5%; neonatal teeth, persistence of primary teeth, delayed eruption), position (62-64%; malocclusion, malalignment, crowded teeth, cross bite), and abnormal tooth shape including talon cusps, a diagnostic hallmark for RTS,[61,115,116]. Talon cusps are accessory cusps on the lingual side of incisors. *CREBBP* and EP300 are strongly expressed in both incisors and molars[117] and influence the formation of the secondary and (to a lesser extent) primary enamel knots, allowing, if mutated, for talon cusp formation in 27% of primary incisors and 70-92% of permanent (upper) incisors,[115,116]. Sealing the fissures around the talon cusps may prevent caries. Treatment is only needed if interfering with mouth closure and occlusion or leading to marked caries (**R40**).

Dental anomalies may also be secondary, i.e. difficulties in maintaining adequate oral health leading to caries and periodontal disease, and also to enamel demineralization due to gastroesophageal reflux,[115,116]. Children and adults with RTS often demonstrate also anxieties when facing dental assessments and treatments, stressing the need of early intervention,[118]. Informing parents and other caregivers of the importance of early adequate oral hygiene, and subsequent advice, is paramount. Regular dental evaluation and treatment, preferably by a dentist with experience in caring for individuals with special needs, can prevent further problems, and treatment may be aided with sedation or general anesthesia,[119] (**R41**). Orthodontic assessments and treatments are as in the general population. However, some

procedures may not be well tolerated and should be considered in close collaboration with the individual and family.

## 14. Immunology

### *14.1. Infections*

Recurrent infections of organs or organ systems do not typically occur in RTS, except for respiratory infections (70% of children, <20% of adults), including otitis media,[35,42,61]. Explanations include microaspiration and gastroesophageal reflux, but dysfunction of the immune response may also contribute. B cell defects have been reported,[62]. If a child with RTS has recurrent unexplained infections, a baseline immune workup including complete blood count (CBC) with differential, immunoglobulin (Ig) levels (IgG, IgA and IgM), vaccine titers and lymphocyte subsets with B cell phenotyping should be performed (**R42**). In lower airway infections microaspiration or gastroesophageal reflux should be considered (**R19**). If the immune workup yields abnormal results, consultation with an immunologist is indicated (**R42**). Although a reduction of T cell or specific T cell subtypes has been found in some cases, combined immune defects such as viral or opportunistic infections, have not been reported and specific antiviral or antifungal prophylaxis is not indicated,[62]. Vaccination can be performed as in the general population, causing the typical level of protection (**R43**).

### *14.2. Oncology*

CREBBP and EP300 are involved in a number of basic cellular activities, such as DNA repair, growth, differentiation, apoptosis, and tumor suppression. Early surveys suggested an increased frequency of malignancies in case reports on individuals with RTS,[120]. However, a more a recent population-based study found no evidence for an increased risk for malignancies in individuals below 40 years of age,[93]. Data for older individuals are too limited to allow conclusions. Benign tumors, however, were more common: meningiomas and pilomatricomas were present in 8% and 17% of molecularly proven patients, respectively,[93]. Surveillance for malignancies below 40 years of age is not recommended; the value of additional surveillance at an older age remains uncertain, and these individuals should follow surveillance schemes according to national standards (**R44**).

## 15. Neurology

### *15.1. Central Nervous System anomalies*

The most common intracranial malformations (74%) in individuals with RTS are corpus callosum–(CC) related malformations. Periventricular posterior white matter abnormalities (63%), cerebellar vermis malformations (58%) and small or absent olfactory bulb (32%) are also regularly observed,[28,54,121–124]. Infrequent findings are Arnold Chiari malformation, underdeveloped pituitary gland, and Dandy-Walker malformation,[28,35,40,41,54,55,125,126]. None of these findings has direct consequences for regular medical care and routine cerebral brain MRI is not recommended and indications for brain MRI studies should follow the standard of care for the general population (**R45**), with the exception of microcephaly without other neurological manifestations. Spinal cord malformations such as tethered cord, syringomyelia, lipomas and spina bifida have also been observed,[13,35,121,124,127]. Spinal MRI is indicated if neurological signs or symptoms are present. Studies for genotype – brain phenotype association haven suggested an association of microcephaly and low-positioning of the conus with an altered KAT function,[121] and no other association.

*15.2. Epilepsy*

Nonspecific electroencephalogram (EEG) abnormalities are observed around 58-76% of individuals with RTS2 but clinical epileptic manifestations are infrequent, ranging from 9-33%,[5,13,57,121,128–130]. In individuals with RTS type 2, epilepsy is reported in 0-10%,[5,13]. Specific EEG findings also in individuals without a history of seizures have been suggested,[121,122], but have no consequences for medical care. Routine EEGs are therefore not recommended, and EEGs should remain limited to individuals with RTS with epileptic seizures. Treatment and surveillance should follow national standards of care. (**R46**).

## 16. Neurodevelopment

The early symptoms of the delayed development are the delay in achieving basic motor skills (Table 3),[35,131]. First words are typically spoken at 2 years of age, sentences of two- or three-words at 4 years of age or later on, with a wide variability across individuals. Intelligence Quotient (IQ) ranges from 25 to 79, nonverbal performance IQ generally being higher than verbal IQ,[41,121,132,133]. Individuals with a *CREBBP* variant typically have a moderate to severe intellectual disability (ID), while individuals with *EP300* variants have mainly a mild ID and only rarely severe ID,[5]. There is no correlation between the type and site of variants and cognitive abilities,[5,11].

Intellectual disability involves related impairments of cognitive function, learning attainment, expressive language, symbolic play and adaptive behavior. The role of reduced neuronal histone acetylation in the etiology of ID has been pointed out by mouse models of

RTS showing deficits in long term memory (LTM), but not in short term memory (STM) upon a variety of learning and memory tasks,[134,135]. Weaker memory impairments were found in *Ep300* mutant mice[136] in keeping with the milder ID of *EP300*- compared to *CREBBP*-mutated individuals. Consolidation of learned information into long term memories through stimuli-driven transcription is mainly imputed to CBP given its interaction with CREB, a key transcription factor involved in memory formation which diminished levels impair spatial memory,[137] as observed in RTS children. Mice with *Cbp* mutation(s) disrupting CBP-CREB interaction, besides memory deficits exhibit impaired motor skill learning,[138] similar to the difficulties in planning and executing motor acts experienced by *CREBBP*-mutated patients.

Early assessment of cognitive abilities will benefit each child to access care earlier and for optimal stimulation of development (**R47).** Non-verbal children may benefit from non-symbolic communication, such as non-speech vocalization and gestures, which helps them in their social interactions, and augmentative communication should be prioritized from early on, also in the preverbal stage (**R48**). Early physiotherapy may enhance rehabilitation as well, focusing on their most weakened skills, which have been identified as those requiring a high level of visuo-motor coordination,[110]. Early implementation and maintenance of communication strategies to catalyze preverbal and verbal language development and socialization skills. Follow-up should include also repeated neuropsychological testing to ensure continuous optimal stimulation, especially at sensitive life phases (school entry, puberty, traumatic events, adulthood and aging),[42] (**R49**).

## 17. Behaviour

### *17.1. Recommendations for clinical practice*

Interventions for behaviours, cognition and emotion specifically for individuals with RTS are lacking. Applying strategies and intervention approaches designed for individuals with intellectual disability in general, as well as interventions for individuals with a diagnosis of autism, may be helpful (Table S4 summarises key recommendations).

### *17.2. Self-injurious and aggressive behaviour*

The prevalences of self-injurious and aggressive behaviour vary markedly in children and adults with RTS (between 7-48% and 10-16%, respectively),[18,139]. These figures are similar to the prevalences in individuals with intellectual disability and autism in general,[140]. Aggressive behaviours may increase in older individuals,[132,141]. Our joint experience indicates that the self-injurious behavior and aggression do not show specific characteristics.

However, formal studies assessing individuals over time and describing specific topographies of behaviour using standardised measures, are lacking.

*17.3. Emotions*

Emotional outbursts, often severe and weekly, were noted in 7/31 children,[139]. However, a questionaire study measuring 'temper tantrums or hot temper' found no differences between children with RTS and typically developing children,[142]. Emotional outbursts were reported in 5/13 adults with RTS,[139] seemingly indicating an increase with age, as reported by others,[132].

On the Child Behaviour Checklist, 64.5% of individuals above 13 years of age and 27.5% of younger individuals were reported to be very anxious,[141]. The anxiety is not correlated with genotypes,[59]. For some anxiety subtypes, scores did not differ from children diagnosed with an anxiety disorder,[143]. Screening for anxieties using a questionnaire validated for individuals with intellectual disability will benefit many individuals with RTS (**R50**). Subsequent interventions should follow best practice guidance for individuals with intellectual disability.

*17.4 Repetitive behaviours*

Repetitive behaviours in individuals with RTS include body, hand and object stereotypy, adherence to routines, repetitive phrases and repetitive questioning,[66,142,144]. Repetitive behaviour, in particular repititive questionning has been associated with inhibitory control and working memory difficulties,[145,146] which has led to the hypothesis that individuals may have difficulties supressing questioning behaviour, and retaining information in their working memory,[145,146]. Co-occurrence of adherence to routines and temper outbursts in older individuals has led to the suggestion that executive function difficulties may contribute to these characteristics,[142,146].

*17.5. Autism Spectrum Characteristics*

Prevalence rates of autism range from 37-44% on standardised screening assessments,[139,142]. The estimates for individuals with a *CREBBP* variant haven been higher (49%) compared to those with an EP300 variant (25%),[5]. Studies utilising direct assessments of children with a *CREBBP* variant and a severe intellectual disability, demonstrate areas of cognitive and socio-emotional differences similar to those in children with a diagnosis of autism matched for degree of disability,[133]. Therefore, families can make use of strategies designed for autism populations, specifically with respect to strategies for language delays, imitation, and symbolic activities,[42] (**R51).**

Caregivers need to be aware that most screening questionnaires use both repetitive behaviour and social behaviour in their scoring, and individuals with RTS may reach the cut-off for autism only because of their repetitive behaviour.

*17.6. Social characteristics*

Social behaviour is typically characterised by motivation to interact with others, and enhanced social skills,[142] and 'over-friendliness' have been reported in >70% of individuals,[132,139] while other studies using observational measures, have suggested social motivation is aligned with typical development,[143].

Parents have reported that their children are vulnerable to social exploitation, particularly as they age and gain independence,[147]. While social motivation is likely to be heightened or preserved, social understanding (e.g. the ability to think about what another may be thinking) is a relative weakness,[147]. Individuals with RTS may benefit from learning appropriate skills to manage complex social situations, understand others' intentions, and reduce impulsivity (**R52**).

*17.7. Self-regulation, impulsivity, and overactivity*

Distractibility, impulsivity, and overactivity have been noted from early descriptions of RTS,[1,35,41]. A short attention span was found in 76-90%,[35,41], irrespective the cognitive level,[142]. Studies yielded varying results regarding hyperactivity, and sometimes underactivity was noticed,[1,35,61,147].

*17.8 Increased pain treshold*

Our joint experience indicates that many parents report their child has not shown evidence of pain or discomfort following a fall or an accident, even for gallstones, fractures, burns or other significant injuries and illnesses. Consequently, it is important not to underestimate subtle changes in behaviour. Medical professionals should be receptive to parent reports, and investigate pro-actively, even if the presence of a major health problem seems unlikely.

## 18. Adult Care

Over 90% of individuals with RTS reportedly survive to adulthood,[71] and progress in diagnostics, knowledge and management abilities allows improved care for older individuals,[61]. Adults with RTS enjoy both social and occupational activities and show a varied experience of everyday life. A recently reported cohort of adults underscored the importance of continued management and follow-up,[42]. Half of all individuals required multi-specialist follow-up and surgery during adulthood, usually more than once. Fortunately,

significant morbidity in adulthood is not frequent. The adult natural history of RTS is defined by behavioural/psychiatric problems (83%), gastrointestinal problems (73%), skin and adnexa problems (65%), sleep problems (62%), and further concerns of high pain threshold, decreased mobility, hypersensitivity to noise and crowded places and vision difficulties or loss (approximately 50%).

The behavioural pattern remains broad but includes frequently rigid, repetitive and inflexible behaviours and emotional dysregulation (anxiety, aggression, frustration and/or a mood disorder) with reported age-dependent progression,[141,144]. Sleep problems show a consistent pattern of sleep apnoea, difficulty staying asleep and an increased need for sleep,[42].

Clinical concerns include gastrointestinal problems with highest frequency of constipation and in much lower frequency, other problems including eosinophilic esophagitis. Retinal dysplasia increases with age,[72] but does not cause severe loss of vision. Skin problems are variable but typically progressive, such as keloid formation, ingrowing finger- and/or toenails (with infections) and poor wound healing,[42]. Hypertension, overweight, diabetes mellitus and cardiovascular problems do occur in adults but in a lower frequency compared to the general population,[42]. Treatment is as in the general population (**R53**).

Data on fertility are limited but likely fertility is not impacted. Adults with RTS may be sexually active (25%),[42]. Risk to offspring is 50% with each pregnancy and familial recurrence has been reported. Thus, developmentally appropriate sexual education throughout the lifespan and especially at transition to adulthood is indicated,[43,61] (**R54**). Contraceptive options should be discussed with the individual and family.

Reliable data on other adult problems such as dementia are not available.

## 18. *Clinical trials*

CBP and p300 have multiple actions and functions, and clinical trials are aimed at decreasing or correcting abnormal functioning. Prenatally, variants in *CREBBP*/*EP300* can cause malformations unamenable to postnatal change (**R55**). Variants can also cause dysplasias, and these may still be influenced postnatally. CBP/p300 are the ‘master co-activators’ of transcription in humans,[148] due to their involvement in many important pathways related to development and differentiation, and postnatal functions such as calcium signalling, nutrient metabolism, hypoxia and stress response,[149–151]. The latter may be influenced postnatally, thus obvious candidate dysfunctions are memory problems, behaviour, keloids, and gastrointestinal problems (**R56**).

### *18.1. Cognition*

*CREBBP/EP300* mutations cause epigenetic modifications that impact brain development and postnatal brain function of *cbp+/cbp-* mice,[150]. Histone deacetylases inhibitors (HDACi) lead to an increase in the acetylation in mice. The HDACi suberoylanilide hydroxamic acid and trichostatin A have been shown to influence neurological functioning and long-term memory in mice,[135].

Inhibitors of phosphodiesterase 4 (PDE4) prevent the hydrolysis of cAMP enhancing PKA-dependent signalling upstream of *CREBBP*. The PDE4 inhibitor rolipram abolishes the long-term memory defects of *cbp+/cbp-* mice,[152]. Rolipram is currently tested in Fragile X syndrome and Alzheimer disease (ClinicalTrials.gov Identifier: NCT03817684) that may be associated with reduced histone acetylation,[153]. If successful it is a candidate to be used in individuals with RTS as well.

The HDAC inhibitor sodium valproate can pass the blood brain barrier. A monocentric, double-blind, randomized, phase 2 trial, primary endpoint long-term memory, investigated the efficiency of sodium valproate after one year of treatment (30 mg/kg/d) in 41 children with RTS (ClinicalTrials.gov NCT01619644). Results using subtests of a neuropsychological test battery specifically designed for memory evaluation did not demonstrate a significant difference between the verum and placebo group. As side effect a slight amelioration of some motor functions was found, and a trial with sodium valproate using motor skills as primary outcome should be considered.

### *18.2. Keloids*

Keloids develop most likely following an inciting stimulus (environmental factor) in genetically predisposed individuals. The unremitting accumulation of thick fibers of collagen I and III in the extracellular matrix of connective tissue places keloids among fibrotic disorders. Keloids are unique to humans, there are no adequate animal models, and a high inter-and intra-lesional heterogeneity impair comparison of *in vitro* models,[87].

The principal cell type responsible for keloids is the myofibroblast derived from resident skin fibroblasts through trans-differentiation or pluripotent stem cells,[154] but also keratinocytes play a distinct role based on their stemness signature,[155]. Fibroblasts from keloids overexpress transforming growth factor (TGF)-β1/2 and their receptors that interact with intracellular SMADs, stimulate transcription of genes intervening in wound healing, and cause persistent inflammation through continuous cell division, growth of extracellular matrix beyond the wound boundary, and abnormal vascularization. Inhibition of the TGF-β1/2 signalling pathway is therefore the main target of keloids therapeutics. Indeed, the TGF-β receptor inhibitor LY2109761 has been shown to suppress secretion of keloid matrix components and to slow down proliferation of derived fibroblasts,[156].

Within keloids several pathways are dysregulated epigenetic modifications including DNA methylation, histone modification and non-coding RNAs,[157,158]. Reverting these epigenetic anomalies to those of normal skin may also lead to successful treatment. Mutated CBP/p300 causes abnormal histone acetylation which may cause the epigenetic signature of keloids in individuals with RTS to be different from that of keloids from individuals with other disorders. Much of the work on histone modifications on keloids has been focussed on the use of the HDAC inhibitor trichostatin A,[159]. Increase in keloids of HDAC2 (and not of other HDACs),[160] suggests topical application of an HDAC2 inhibitor to be a potential treatment,[157]. CUDC-907 is an inhibitor of HDAC and also of the PI3K/AKT/mTOR pathway, and has been proposed as candidate systemic drug,[161].

Another approach is using upregulation of the mitochondrial oxidative stress response and protein processing in the endoplasmic reticulum (ER),[162]. Treatment with an inhibitor of ER stress tauroursodeoxycholic acid (TUDCA) reduced scar formation in the rabbit ear,[162]. The potential use in man is favored by the clinical approval of TUDCA in cholestasis, and its effective inhibition of ER stress in fibropulmonary disease in mice,[163]. Single cell RNA sequencing of keloid tissue has shown significant expansion of fibroblast and vascular endothelial cell subpopulations, responsible for the aberrant keloid fibrogenesis and angiogenesis. In fibroblasts *TWIST1* and *SMAD3* are top upregulated genes and TWIST1 inhibition has been proposed as therapeutic target [Liu 2021]. Tumour-related pathways are activated in fibroblast and endothelial cell subpopulations, accounting for the excessive proliferation and resistance to apoptosis of keloids,[164] and indicating transferability and efficiency of medical therapies applied in tumors for the clinical treatment of keloids.

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**Competing interests**

The authors declare no competing interests.

**Ethics approval statement**

The authors affirm that human research participants provided informed consent, for publication of the images in Figure 1.

**Contributorship statement**

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## Tables

**Table 1**. Main clinical findings in percentages of individuals with molecularly confirmed Rubinstein-Taybi syndrome.

| | HPO ID[1] | *CREBBP* (n=308) | *EP300* (n=52) |
|---|---|---|---|
| ***Growth*** | | | |
| Intrauterine growth retardation | 0001511 | 49 | 42 |
| Postnatal growth retardation | 0004322 | 75 | 66 |
| Obesity | 0001513 | 29 | 39 |
| Microcephaly | 0000252 | 54 | 87 |
| ***Craniofacial features*** | | | |
| Highly arched eyebrows | 0002253 | 85 | 65 |
| Long eyelashes | 0000527 | 89 | 90 |
| Epicanthal folds | 0000286 | 44 | 15 |
| Strabismus | 0000486 | 71 | 39 |
| Myopia | 0000545 | 56 | 24 |
| Downslanted palpebral fissures | 0000494 | 79 | 56 |
| Convex nasal ridge | 0000444 | 81 | 44 |
| Columella below alae nasi | 0009765 | 88 | 92 |
| Typical smile[2] | 0000273 | 94 | 47 |
| Highly arched palate | 0002705 | 77 | 67 |
| Talon cusps[3] | 0011087 | 73 | 4 |
| Micrognathia | 0000347 | 61 | 42 |
| Low-set ears | 0000369 | 44 | 27 |
| ***Trunk and limbs*** | | | |
| Broad thumbs | 0011304 | 96 | 69 |
| Angulated thumbs | [4] | 49 | 2 |
| Broad finger tips | 0011300 | 87 | 22 |
| Broad halluces | 0010055 | 95 | 81 |
| Hypertrichosis | 0000998 | 76 | 51 |
| Keloids | 0010562 | 23 | 10 |
| Scoliosis | 0002650 | 18 | 25 |
| Cardiovascular anomalies | 0002564 | 35 | 26 |
| Constipation | 0002019 | 76 | 54 |
| Urinary tract anomalies | 0000079 | 28 | 24 |
| ***Neuromuscular*** | | | |
| Seizures | 0001250 | 25 | 10 |
| ***Cognition and behaviour*** | | | |
| Intellectual disability (any degree) | 0001249 | 99 | 94 |
| Autism/Autism spectrum disorder | 0000729 | 49 | 25 |

[1] HPO ID, Human Phenotype Ontology Identifier; [2] Smile characterized by crescent-moon shaped palpebral fissures, deepening of labionasal folds, upturned corners of the mouth, usually mouth almost closed, tight upper vermillion and pouting lower vermillion; [3] Permanent dentition; [4] no HPO identifier available; we used as definition: angulation of the distal phalanx of a thumb towards the anterior axis (radial side) of the limb

**Table 2.** Clinical diagnostic criteria for Rubinstein-Taybi Syndrome

| **Cardinal** | **Supportive** |
|---|---|
| 1. Face (at least three of six) | a. Maternal preeclampsia |
| a. Highly arched eyebrows | b. Keloids |
| b. Downslanted palpebral fissures | c. Hypertrichosis |
| c. Convex nasal ridge | **1point** if *c* is positive, or |
| d. Columella below alae nasi | **3 points** if *a* and/or *b* (with or without *c*) are positive |
| e. Highly arched palate | |
| f. Typical smile | |
| **3 points** or | |
| **4 points** if *d* and/or *f* are positive | |
| 2. Skeletal | |
| a. Angulated thumbs and/or halluces | |
| b. Broad thumbs | |
| c. Broad halluces | |
| **3 points** if *b* and/or *c* is positive or | |
| **4 points** if *a* (with or without *b/c*) is positive | |
| 3. Growth | |
| a. Microcephaly | |
| b. Postnatal growth retardation | |
| **2 points** if *a* and/or *b* are positive | |
| 4. Development | |
| Delayed development / Intellectual disability | |
| **2 points** | |

**Definitive clinical diagnosis of Rubinstein-Taybi syndrome:**

**Score ≥ 12** and positive cardinal score.

**Likely clinical diagnosis of Rubinstein-Taybi syndrome**

**Score 8-11** and positive cardinal score. This score warrants molecular analyses of *CREBBP* and *EP300.*

**Possible clinical diagnosis of Rubinstein-Taybi syndrome**

**Score 5-7** and negative cardinal score. This score warrants molecular analyses of *CREBBP* and *EP300.*

**Unlikely clinical diagnosis of Rubinstein-Taybi syndrome**

Score 0-4 and negative cardinal score. Further studies for other aetiologies indicated.

**Table 3.** Developmental milestones of children with Rubinstein–Taybi syndrome compared with typically developing children.

| | Rubinstein–Taybi syndrome | | General population (Dowman 2012) | |
|---|---|---|---|---|
| **Milestone** | **Mean age (months)** | **Range** | **Mean age (months)** | **Range** |
| Laughing | 2.5 | 2–6 | 2 | 2–6 |
| Rolling over | 10 | 4–18 | 6 | 5–9 |
| Sitting | 16 | 9–24 | 7 | 6–12 |
| Crawling | 19 | 12–36 | 9 | 8–12 |
| Standing | 29 | 11–80 | 9 | 8–18 |
| Walking | 35 | 18–54 | 14 | 12–18 |
| First words | 24 | 6–84 | 12 | 8–18 |

**Figure legends:**

**Figure 1.** Cardinal features of the clinical diagnostic criteria of face and limbs for Rubinstein-Taybi syndrome (RTS).

**Figure 2. Structures and Functions of CBP/p300.**

**A.** The proteins CBP and p300 are composed of 2442 amino acids (AA) and 2414 AA, respectively, with 58% of sequence similarity within their domains. The various domains are represented with their position in the AA sequence: N-terminal nuclear receptor interaction domain (NRID or RID), cysteine-histidine rich region 1 (C/H1) containing the transcriptional adapter zinc finger 1 (TAZ1), kinase-inducible domain (KID) interacting domain (KIX), Bromodomain, C/H2 containing a plant homeodomain (PHD), Lysine acetyltransferase domain (KAT), C/H3 containing the zinc finger (ZZ) and TAZ2 domains, and interferon-binding transactivation domain (IBiD). The MKHKS region corresponds to the location of the missense variants leading to the Menke-Hennekam syndrome.

**B**. CBP and p300 act as transcriptional co-activators of target genes by different mechanisms: (1) Binding function by facilitating the physical and functional interactions of TF; (2); Scaffolding function allowing the recruitment of TF and in particular CREB (3) KAT function by catalyzing the transfer of acetyl groups on lysine residues of both histone tails and non-histone proteins such as the RNApolII complex and TF. TBP: TATA binding protein; TF: transcription factors; Ac: acetyl group. Adapted from Van Gils *et al.* 2021,[15].

**Figure 3. Mutation spectrum of *CREBBP* and *EP300* in individuals with RTS (referenced in HGMDPro variant database and/or LOVD).**

**A.** Repartition of 500 pathogenic variants in *CREBBP* referenced as causing RTS1 including 84 nonsense variants, 192 frameshift variants, 46 splicing variants, 84 missense variants, 75 intragenic deletions, 14 deletions including *CREBBP* completely, 2 intragenic duplications and 3 complex rearrangements.

**B.** Repartition of 118 pathogenic variants in *EP300* referenced as causing RTS2 including 26 nonsense variants, 56 frameshift variants, 6 splicing variants, 16 missense variants, 11 intragenic deletions and 3 deletions encompassing *EP300* completely. Adapted from Van Gils *et al.* 2021,[15].

**Figure 4. Molecular diagnostic pathways for Rubinstein-Taybi syndrome.** In individuals with clinically classic RTS phenotype, the first-line molecular diagnostic approach is targeted analysis of *CREBBP* and *EP300* by Sanger sequencing and MLPA or by high throughput

analysis (aCGH; WES). In individuals in whom RTS is not suspected, aCGH and WES or WGS is performed. [a] Including analysis of *CREBBP* / *EP300* and genes causing related entities; [b] Evaluation of results using ACMG classification,[23]; [c] Episignature specific for RTS,[24]; [d] RNA studies; searches for mosaicism.