Rapid Communication

Disruption of the CREBBP gene and decreased expression of CREB, NFκB p65, c-JUN, c-FOS, BCL2 and c-MYC suggest immune dysregulation

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ABSTRACT

Genomic aberrations in the *CREBBP* (CREB-binding protein – *CREBBP or CBP*) gene such as point mutations, small insertions or exonic copy number changes are usually associated with Rubinstein-Taybi syndrome (RTs). In this study, the disruption of the *CREBBP* gene on chromosome 16p13.3, as revealed by CGH-array and FISH, suggests immune dysregulation in a patient with the Rubinstein Taybi syndrome (RTs) phenotype. Further investigation with Western blot techniques demonstrated decreased expression of CREB, NFκB, c-Jun, c-Fos, BCL2 and cMyc in peripheral blood mononuclear cells, thus indicating that the *CREBBP* gene is essential for the normal expression of these proteins and the regulation of immune responses.

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1. Introduction

The *CREBBP* or *CBP* gene, which is located on chromosome 16p13.3, codes for a multidomain protein essential in embryonic development, growth control and homeostasis by coupling chromatin remodeling to transcription factor recognition [33]. The *CREBBP* gene was first described by Chrivia et al. [12] as a transcriptional coactivator. Its nomenclature is based on the interaction of the CREB protein with nuclear *CREBBP* [12]. The *CREBBP* gene has 31 exons, occupies approximately 159 kb of genomic DNA and encodes a protein of 265 kDa with 2442 amino acids [18]. *CREBBP* gene mutations lead to inactivation of one allele in patients with Rubinstein Taybi syndrome (RTs) [40,41]. The *CREBBP* gene appears to be crucial at certain stages of embryonic development, which could explain all the phenotypic changes that characterize RTs patients [19,20]. Most of the conserved domains of CREBBP function to bind a diverse array of proteins, including more than 50 vital T-cell transcriptional regulators [30]. Originally, the *CREBBP* gene was found to act as a physical link between DNA-binding transcription factors.

Furthermore, *CREBBP* facilitates transcription through intrinsic histone acetyltransferase (HAT) activity, which opens up condensed DNA and thereby allows greater access to the transcriptional machinery. CREBBP has been shown to interact with CREB, c-Jun, NF-κB and c-Fos. All of these transcription factors play a critical role in regulating the expression of many inflammatory and immune response genes [5,27,17,39,6]. Studies have shown the involvement of CREBBP in regulating the immune response and cell differentiation; failure of the gene leads to the formation of benign and malignant tumors and hematologic malignancies [4,54,11].

RTs usually occurs sporadically, although it can be inherited as an autosomal dominant disorder (OMIM 180849) [21,22]. The diagnosis is still essentially clinical and based on characteristic features [22]. The major items to look for in RTs are a beaked nose, grimacing smile, broad thumbs and big toes, and mental retardation [23]. The main clinical problems are failure to thrive, congenital heart defects, and recurrent respiratory infections [48]. *EP300*, a gene located on chromosome 22q13.3, has been identified as another gene involved in causing RTs [43,44]. Mutations in the *EP300* gene in RTs patients are rare and have only been found in eight individuals with clinical signs of incomplete or non-classical syndrome [7,58,9,16,51].

We performed an immunological screening of the major mechanisms of adaptive and innate immune response in a patient with an apparent balanced translocation involving chromosomes 2 and l6, previously described by Torres et al. [49,50], and clinical features of RTs. Further investigations using CGH-array, FISH and Western blot techniques revealed a disruption of the *CREBBP* gene that resulted in decreased expression of the CREB, NFκB p65, c-JUN, c-FOS, BCL2 and c-MYC proteins in peripheral blood mononuclear cells.

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2. Materials and methods

2.1. Clinical report

The patient is the third child of healthy unrelated parents. She was born pre-term by cesarean section; her birth weight was 1900 g (below the 5th percentile), her length was 47 cm (5th – 10th percentile), and her head circumference was 36 cm (75th percentile). Her global development was delayed: she could sit without support at 13 months, walk unassisted at 2.5 years, had toilet control at 4 years and she spoke her first meaningful words at 18 years. She had frequent feeding problems during her first five years of life. Splenomegaly was noted at 21 years of age.

At 23 years of age, the patient's weight was 61 kg (50th – 75th percentile), her height was 139.5 cm (below the 3rd percentile) and her OFC was 49.5 cm (below the 3rd percentile). She was found to have highly arched eyebrows, down-slanting palpebral fissures, a beaked nose, a *nasal septum* that extended *below* the *alae* nasi, a highly arched palate and *posteriorly* angulated *ears*. She also had broad, radially deviated thumbs and broad halluces. She presented mild leukocytosis (11,000–13,000/mm^3) despite the absence of clinical signs of infection. A high rate of B cell apoptosis and a 40–50% decrease of the total polymorphonuclear leukocyte count with dysgranulopoietic granulocytes were also observed by hemogram.

2.2. DNA isolation

Blood samples from the patient and her parents were collected with vacuum-EDTA after informed consent (approved by the Research Ethics Committee of HC-FMUSP CAPPESQ number 1158/07). DNA extraction was carried out using the Qiagen QIAamp® DNA blood mini kit (Valencia, CA, USA).

2.3. Western blot analysis

Peripheral blood mononuclear cells (PBMCs) were isolated from one RTs patient and controls. Total protein was extracted using a PARIS™ Kit (Applied Biosystems, Ambion, USA) and a mixture of protease inhibitors according to the manufacturer's protocol. Protein concentration was determined using a Nanovue spectrophotometer (GE Healthcare, Piscataway, NJ, USA).

Protein samples from each subject were individually loaded (60 μg per lane) onto 10% acrylamide gels, resolved by electrophoresis, and transferred onto nitrocellulose membranes. Membranes were blocked with 5% milk and subsequently incubated overnight at 4 °C with the following primary antibodies: anti-CREB (1.0 μg/mL), anti-cFOS (0.2 μg/mL), anti-cJUN (0.5 μg/mL), anti-NFκB p65 (0.5 μg/mL), anti-BCL2 (1.0 μg/mL) and anti-cMYC (0.5 μg/mL). All the antibodies used in this study were obtained from Chemicon (Millipore, USA). Primary antibodies were detected by horseradish peroxidase (HRP)-conjugated secondary antibodies. Membranes were developed using Luminol Western Blotting Substrate according to the manufacturer's instructions (Millipore, USA) and detected by chemiluminescence. The obtained bands were quantitated and photographed in the LAS 4000 (Fujifilm, SA) using *Image 4000 software* (Fujifilm, SA).

3. Molecular cytogenetic analysis

3.1. Array-CGH studies

Agilent Human Genome CGH 244A microarrays (Agilent Technologies, Santa Clara, CA, USA) containing unique oligonucleotides representing 244,000 probes with an average probe spacing across the human genome of 6.4 Kb were used for the CGH-array experiments. Human genomic DNA from multiple anonymous male donors was obtained from Promega Corporation (Madison, USA) and used as a control. A microarray assay was performed using the Agilent Human Genome CGH 244A microarray according to the manufacturer's protocol version 2.0 (August 2005) for Oligonucleotide Array-Based CGH for high-throughput whole genomic DNA analysis (Agilent Technologies, Inc., Palo Alto, USA). The array was scanned and analyzed using an Agilent 2565AA DNA microarray scanner (Agilent Technologies, Inc., Palo Alto, USA) and Feature Extraction software, respectively, according to NCBI 36/hg 18 annotations.

3.2. Fluorescence in situ hybridization (FISH)

Blood samples from the patient and her parents were processed by standard cytogenetic procedures. FISH was performed on metaphase cells using different cosmid probes (RT1, RT100, RT102, RT191, RT203, and RT166) that cover the entire *CREBBP* gene except for the ~5 kb uncloned area between RT203 and RT166. A chromosome 16-specific centromeric probe was co-hybridized with probe pHUR195 in accordance with [41], with modifications. FISH with probes RP11-690I21 from 2q37.1 and RP11-90D1 from 2p16.1 was also used to investigate chromosomal breakpoints (probes were obtained from Baylor College of Medicine-Medical Genetics Laboratories, Houston, Texas) [32].

4. Results

4.1. Cytogenetic and molecular findings

The previous GTG-banding chromosome analyses of the patient demonstrated a 46,XX,t(2;16) karyotype, suggesting a balanced translocation. The parents presented normal karyotypes.

Because no apparent deletions in the *CREBBP* gene that could explain the RTs phenotype were identified, CGH-array analysis was performed to investigate the presence of microdeletions or other genomic imbalances not detected by standard techniques such as GTG-banding.

The CGH-array result showed that the patient carried benign copy number alterations, namely, losses of approximately 400 bp at 19p13.3 (794,778–795,178) also present in patient's father, and losses at 16q23 (76,938,523–76,938,923) that contain no genes or evidence in the literature associated with any pathogenic features. The patient's mother also carried a small deletion of approximately 232 bp at 16p13.3 (2,638,381–2,638,612) with no clinical significance [14,26]. Quite surprisingly, microdeletions in the 16p13.3 region were not detected in the patient as expected. Subsequent FISH analysis showed that the translocation revealed in classical karyotype analysis in fact involved the short arms of chromosomes 2 and 16. The corrected karyotype is 46,XX.ish t(2;16)(p11.2;p13.3) (RT100+,RT166−;RT100+;RT166). Therefore, we concluded that the *CREBBP* gene is disrupted by this genomic rearrangement (Fig 1).

4.2. Western blot findings

Western blot analysis showed low amounts of CREB, NFκB p65, AP-1 (c-Jun and c-Fos), Bcl2 and cMyc in the patient's peripheral blood mononuclear cells. Image quantification analysis showed that the expression of these proteins was diminished by 50% in the patient's cells when compared to two healthy controls, as depicted in Fig. 2.
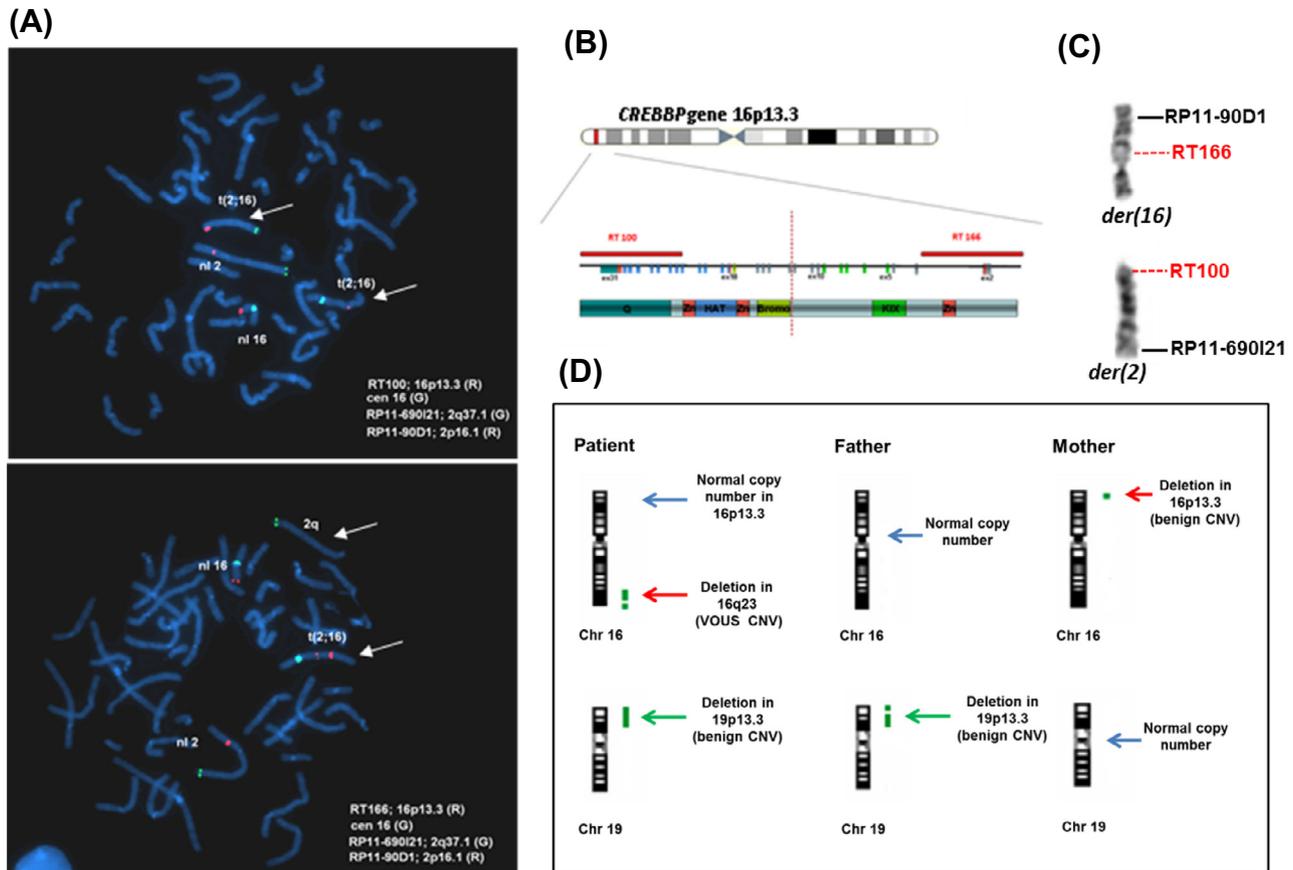
CNVs: Copy number alterations; VOUS: CNV with Uncertain clinical significance; likely benign; Benign CNVs: represents a common polymorphism.

Fig. 1. (A) FISH with probes RP11-690I21 (green signal at 2q37.1), RP11-90D1 (red signal at 2p16.1), RT100 and RT166 (red signals at 16p13.3). The arrows indicate the derivative chromosomes. (B) Ideogram showing the RT100 and RT166 probes positions in *CREBBP* gene. (C) Images of the derivative chromosomes 2 and 16 in G band showing probes hybridization positions. (D) Ideogram showing CGH-array results and CNVs interpretation according Miller et al, 2010 [14].

5. Discussion

Most patients with RTs present with recurrent respiratory tract infections and demonstrate elevated susceptibility to infections during infancy and childhood. It was thought that these infections were due to structural abnormalities, microaspiration, and/or gastroesophageal reflux ([36]).

In the present study, we demonstrated that the disruption of one *CREBBP* allele due to a chromosomal translocation affected a subset of CREBBP-interacting factors and led to reduced production of NFκB, c-Jun, c-Fos, Bcl2 and c-MYC in the patient's mononuclear cells.

Thus far, the effects of *CREBBP* gene mutations on the RTs phenotype could only be studied in animal models of RTS. *CREBBP* and *EP300* knockout mice or mutant heterozygote mice for both genes die as embryos. In addition, loss of *CREBBP* and *EP300* is highly deleterious to T and B lymphocytes in mice [37,52,31].

The activity of some transcription factors, such as c-JUN, c-FOS, and c-MYC, is regulated by their interaction with *CREBBP* transcription coactivators. The NFκB, c-Jun, c-Fos, Bcl2 and c-MYC were shown to be active in several animal models of inflammation and immunosupression [10,13,24,29,35,42].

Furthermore, in transgenic mice that have a dominant inhibitory form of CREB, the expression of several Activator Protein 1 (AP-1) family members that include cFos and cJun, was specifically reduced in thymocytes, suggesting that CREB plays an important role in regulating the expression of AP-1 by T cells. In human cells, CREB, ATF-2, and c-Jun are recruited to the proximal promoter of IFN-γ and regulate IFN-γ transcription in response to microbial pathogens. Additionally, ATF-2 controls the expression of CREB and c-Jun during T cell activation [8,45]. AP-1 is a heterodimer containing Fos and Jun proteins that can regulate the transcription of multiple lymphokines, chemokines, and cell surface receptor genes, as well as directly facilitate entry of T cells into the cell cycle [46,28]. Moreover, the NF-κB transcription factor participates in the immune response to infection. The incorrect regulation of NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and defects in immune development ([19,20,55]). NF-κB p65 and CREBBP can form two distinct interactions: a phosphorylation-independent interaction that involves the C-terminal region of p65 and the N-terminal region of *CREBBP* and a phosphorylation-dependent interaction that requires phosphorylation of serine 276 of p65 and the KIX region of *CREBBP* [38,57]. Baeuerle et al. showed that IKKβ/NFκB is an essential mediator of the immune response and the inflammatory process [3]. NFκB is major transcription factor in immune cells and is responsible for the production of proinflammatory cytokines and other important factors to the inflammatory response [3].

Some investigators have suggested that recruitment of CREBBP to p65-containing NF-κB complexes is essential for transcriptional activity and that this interaction between p65 and *CREBBP* is an
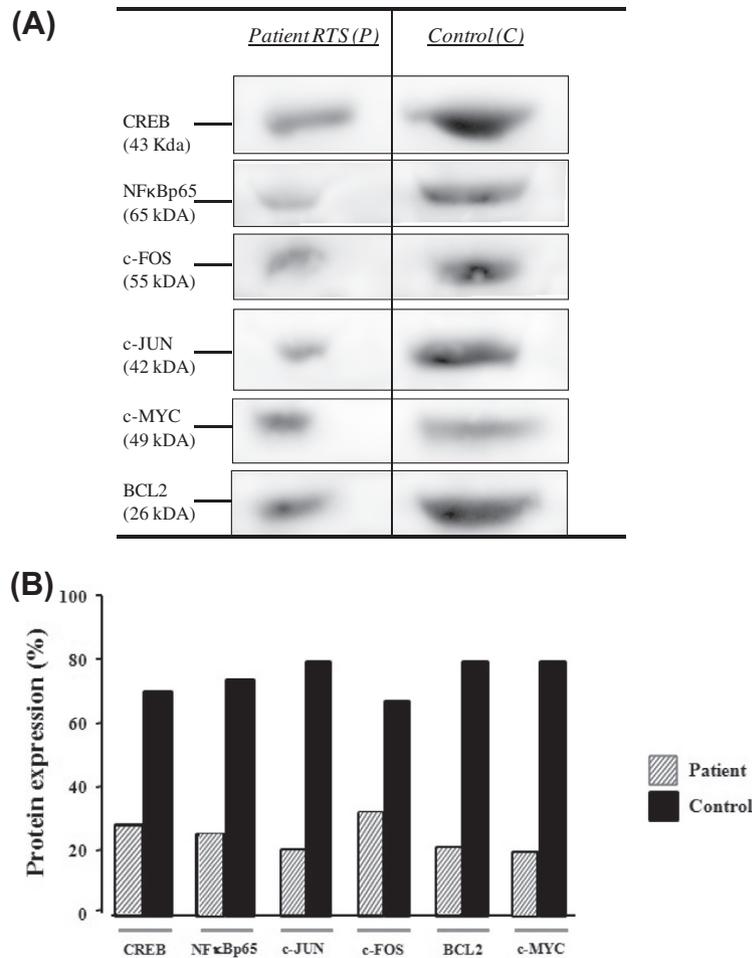
(A)

(B)

Fig. 2. Protein expression of CREB, NFκBp6, c-FOS, c-JUN, BCL2 and c-MYC in PBMC by Western Blot: (A) Images of protein bands onto nitrocellulose membranes from RTS patient (P) and healthy control (C); (B) Percentage of the protein expression performed by ImageQuant TL software.

important requirement for efficient transcription by NF-κB [38,57,45].

Wilson et al. [56] have demonstrated that CREB is an important transcription factor of the BCL-2 gene in B cells and that CREB interacts with the BCL-2 promoter after activation of these cells, enabling the expression of BCL-2, which culminates in the protection of the cells from apoptosis. Thus, BCL-2 has an important role in not only saving the cells from apoptosis but also inhibiting cell proliferation by regulating the expression of another gene called c-MYC [1]. Vaux et al. [53] first demonstrated that c-Myc causes cell death when growth factors are deprived, that Bcl-2 can enhance the survival of c-*myc* expressing cells and that Bcl-2 collaborates with c-Myc to immortalize pre-B cells. The Bcl-2 protein can inhibit apoptosis and cell proliferation by regulating the expression of c-MYC. Arcinas et al. [1] showed that decreased cellular *BCL-2* levels led to increased expression of *c-MYC*. Therefore, the relationship between the effects of the *BCL-2* and *c-MYC* genes on lymphomas was attributed to the ability of Bcl-2 to prevent the apoptosis and cell proliferation induced by c-MYC [15]. The MYC transcription factor is one of the most important somatically mutated oncogenes in human cancer [2,34,47].

Given that the patient represents a natural knockout human model for inactivation of the *CREBBP* gene, we suggest that this gene is essential for normal co-expression of the CREB, NFκB p65, c-JUN, c-FOS, BCL2 and c-MYC transcription factors in peripheral blood mononuclear cells.

The known haploinsufficient genes show higher levels of expression during early development and tissue specificity.

Regarding functional interaction, the human *CREBBP* gene most likely has more interaction partners and complex network proximity to other known haploinsufficient genes. This study indicates that *CREBBP* haploinsufficiency disrupts this interaction mechanism, thereby most likely causing the immune dysregulation observed in RTS [25].

Furthermore, the presence of structural genomic alterations in *CREBBP*, as well as gene dosage imbalance, may affect not only locus but also gene regulation in RTS patients.

References

[1] Arcinas M, Heckman CA, Mehew JW, Boxer LM. Molecular mechanisms of transcriptional control of bcl-2 and c-myc in follicular and transformed lymphoma. Cancer Res 2001;61:5202–6.
[2] Aulmann S, Adler N, Rom J, Helmchen B, Schirmacher P, Sinn HP. C-myc amplifications in primary breast carcinomas and their local recurrences. J Clin Pathol 2006;59:424–8.
[3] Baeuerle PA, Baltimore D. NFkappaB: ten years after 1. Cell 1996;87:13–20.
[4] Baldwin Jr AS. The NF-kappa B and I kappa B proteins: new discoveries and insights review. Annu Rev Immunol 1996;14:649–83.
[5] Bannister AJ, Kouzarides T. The CREBBP co-activator is a histone acetyltransferase. Nature 1996;3:19–26.
[6] Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin Sci 1998;94:557–72.

[7] Bartholdi D, Roelfsema JH, Papadia F, et al. Genetic heterogeneity in Rubinstein – Taybi syndrome: delineation of thephenotype of the first patients carrying mutation in EP300. J Med Genet 2007;44:327–33.

[8] Barton K, Muthusamy N, Chanyangam M, Fischer C, Clendenin C, Leiden JM. Defective thymocyte proliferation and IL-2 production in transgenic mice expressing a dominant-negative form of CREB. Nature 1996;379:81–5.

[9] Bartsch O, Labonté J, Albrecht B, et al. Two patiensts with EP300 mutations and facial dysmorphism different from the classic Rubinstein-Taybi syndrome. Am J Med Genet A 2010;152A(1):181–4.

[10] Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, et al. Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet 2008;40:43–50.

[11] Chen LF, Greene WC. Shaping the nuclear action of NF-kB. Nat Rev Mol Cell Biol 2004;3:392–401.

[12] Chrivia JC, Kwok RPS, Lamb N, Hagiwara M, Montminy MR, Goodman RH. Phosphorylated CREB binds specifically to the nuclear protein. Nature 1993;365:855–9.

[13] Clayton AL, Rose S, Barratt MJ, Mahadevan LC. Phosphoacetylation of histone H3 on c-fos- and c-jun-associated nucleosomes upon gene activation. EMBO J 2000;19:3714–371426.

[14] Miller David T, Adam Margaret P, Aradhya Swaroop, Biesecker Leslie G, Brothman Arthur R, Carter Nigel P, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 2010;86(14):749–64.

[15] Fanidi A, Harrington EA, Evan GI. Cooperative interaction between c-myc and bcl-2 proto-oncogenes. Nature 2004;359:554–6.

[16] Foley P, Bunyan D, Stratton J, et al. Further case of Rubinstein-Taybi syndrome due to a deletion in EP300. Am J Med Genet A 2009;149A:997–1000.

[17] Gerritsen ME, Williams AJ, Neish AS, Moore S, Shi Y, Collins T. CREB-binding protein/p300 are transcriptional coactivators of p65. Proc Natl Acad Sci USA 1997;94:2927–32.

[18] Giles RH, Petrij F, Dauwerse HG, et al. Construction of a 1,2- Mb contig surrounding, and molecular analysis of, the human CREB-binding protein (CBP/CREBBP) gene on chromosome 16p13.3. Genomics 1997;42:96–114.

[19] Goodman RH, Smolik S. CBP/p300 in cell growth, transformation, and development. Genes Dev 2000;14:1553–77.

[20] Goodman RH, Smolik S. CBP/p300 in cell growth, transformation, and development. Genes Dev 2000;14:1553–77.

[21] Hamosh A, Scott AF, Amberger J, et al. Online Mendelian inheritance in man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res 2002;30:52–5.

[22] Hennekam RC, Stevens CA, Van de Kamp JJ. Etiology and recurrence risk in Rubinstein-Taybi syndrome. Am J Med Genet Suppl 1990;6:56–64.

[23] Hennekam RC. Rubinstein-Taybi syndrome. Eur J Hum Genet 2006;14:981–5.

[24] Horvai AE, Xu L, Korzus E, Brard G, Kalafus D, Mullen TM, et al. Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CREBBP and p300. Proc Natl Acad Sci USA 1997;94:1074–9.

[25] Huang N, Lee I, Marcotte EM, Hurles ME. Characterising and predicting haploinsufficiency in the human genome. PLoS Genet 2010;6(10):e1001154.

[26] Kearney HuttonM, Thorland ErikC, Brown KerryK, Quintero-Rivera Fabiola, South SarahT. A working group of the American college of medical genetics (acmg) laboratory quality assurance committee. American college of medical genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. Genet Med 2011 Jul;13(7):680–5.

[27] Janknecht R, Nordheim A. MAP kinase-dependent transcriptional coactivation by Elk-1 and its cofactor CREBBP. Biochem Biophys Res Commun 1996;7:228–831.

[28] Jochum W, Passegue E, Wagner EF. AP-1 in mouse development and tumorigenesis. Oncogene 2001;20:2401–12.

[29] Karsenty G, Wagner EF. Reaching a genetic and molecular understanding of skeletal development. Dev Cell 2002;2:389–406.

[30] Kasper LH, Fukuyama T, Biesen MA, Boussouar F, Tong C, de Pauw A, et al. Conditional knockout mice reveal distinct functions for the global transcriptional coactivators CBP and p300 in T-cell development. Mol Cell Biol 2006;26(3):789–809.

[31] Kasper LH, Thomas MC, Zambetti GP, Brindle PK. Double null cells reveal that CREBBP and p300 are dispensable for p53 targets p21 and Mdm2 but variably required for target genes of other signaling pathways. Cell Cycle 2011;10: 212–21.

[32] Kulikowski LD, Christ LA, Nogueira SI, Brunoni D, Schwartz S, Melaragno MI. Breakpoint mapping in a case of mosaicism with partial monosomy 9p23 > pter and partial trisomy 1q41-qter suggests neo-telomere formation in stabilizing the deleted chromosome. Am J Med Genet A 2006;140(1):82–7.

[33] Liu X, Wang L, Zhao K, Thompson PR, Hwang Y, Marmorstein R, et al. The structural basis of protein acetylation by the p300/**CBP** transcriptional coactivator. Nature 2008;451:846–50.

[34] Marin MC, Hsu B, Stephens LC, Brisbay S, McDonnell TJ. The functional basis of c-myc and bcl-2 complementation during multistep lymphomagenesis in vivo. Exp Cell Res 1995;217:240–7.

[35] Munhoz CD, Sorrellsm SF, Caso JR, Scavone C, Sapolsky RM. Glucocorticoids exacerbate lipopolysaccharide-induced signaling in the frontal cortex and hippocampus in a dose-dependent manner. J. Neurosci 2010;30:13690–8.

[36] Naimi DR, Munoz J, Rubinstein J, Hostoffer Jr RW. Rubinstein-Taybi syndrome: an immune deficiency as a cause for recurrent infections. Allergy Asthma Proc 2006;27:281–4.

[37] Oike Y, Takakura N, Hata A, Kaname T, Akizuki M, Yamaguchi Y, et al. Mice homozygous for a truncated form of CREB-binding protein exhibit defects in hematopoiesis and vasculo-angiogenesis. Blood 1999;93:2771–9.

[38] Parker D, Ferreri K, Nakajima T, LaMorte VJ, Evans R, Koerber SC, et al. Phosphorylation of CREB at Ser-133 induces complex formation with CREB-binding protein via a direct mechanism. Mol Cell Biol 1996;16:694–703.

[39] Perkins ND, Felzien LK, Betts JC, Leung K, Beach DH, Nabel GJ. Regulation of NF-kappaB by cyclindependent kinases associated with the p300 coactivator. Science 1997;275:523–7.

[40] Petrij F, Giles RH, Dauwerse HG, et al. Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature 1995;376:348–51.

[41] Petrij F, Dorsman JC, Dauwerse HG, Giles RH, Peeters T, Hennekam RC, et al. Rubinstein-Taybi syndrome is caused by mutations in the transcriptional co-activator CREBBP. Nature 1995;376:348–51.

[42] Rajabi HN, Baluchamy S, Kolli S, Nag A, Srinivas R, Raychaudhuri P, et al. Effects of depletion of CREB-binding protein on c-Myc regulation and cell cycle G1-S transition. J Biol Chem 2005;280:361–74.

[43] Roelfsema JH, White SJ, Ariyürek Y, et al. Genetic heterogeneity in Rubinstein-Taybi syndrome: mutations in both the CBP and EP300 genes cause disease. Am J Hum Genet 2005;76:572–80.

[44] Roelfsema JH, White SJ, Ariyurek Y, Bartholdi D, Niedrist D, Papadia F, et al. Genetic heterogeneity in Rubinstein-Taybi syndrome: mutations in both the CREBBP and EP300 genes cause disease. Am J Hum Genet 2005;76:572–80.

[45] Samten B, Townsend JC, Weis SE, Bhoumik A, Klucar P, Shams H, et al. CREB, ATF, and AP-1 transcription factors regulate IFN-gamma secretion by human T cells in response to mycobacterial antigen. J Immunol 2008;181:2056–64.

[46] Shaulian E, Karin M. AP-1 as a regulator of cell life and death. Nat Cell Biol 2002;4:131–6.

[47] Smith DP, Bath ML, Metcalf D, Harris AW, Cory S. MYC levels govern hematopoietic tumor type and latency in transgenic mice. Blood 2006;108: 653–61.

[48] Stevens CA, Carey JC, Blackburn BL. Rubinstein-Taybi syndrome: a natural history study. Am J Med Genet Suppl 1990;6:30–7.

[49] Torres LC, de Lourdes Lopes Chauffaille M, Delboni TP, Okay TS, Carneiro-Sampaio M, Sugayama S. Clinics 2010;65:107–9.

[50] Torres LC, Sugayama SM, Arslanian C, Sales MM, Carneiro-Sampaio M. Evaluation of the immune humoral response of Brazilian patients with Rubinstein-Taybi syndrome. Braz J Med Biol Res 2010;43:1215–24.

[51] Tsai A, Dosset C, Walton C, et al. Exon deletions of the EP300 and CREBBP genes in two children with Rubinstein Taybi syndrome detected by aCGH. Eur J Hum Genet 2010;19:43–9.

[52] Valor LM, Pulopulos MM, Jimenez-Minchan M, Olivares R, Lutz B, Barco A. Ablation of CREBBP in forebrain principal neurons causes modest memory and transcriptional defects and a dramatic reduction of histone acetylation but does not affect cell viability. J Neurosci 2011;31:1652–16563.

[53] Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 1988;335: 440–2.

[54] Villella A, Bialostocky D, Lori E, et al. Rubinstein-Taybi syandrome with humoral and cellular defects: a case report. Arch Dis Child 2000;83:360–1.

[55] Vo N, Goodman RH. CREB-binding protein and p300 in transcriptional regulation. J Biol Chem 2001;276:13505–8.

[56] Wilson BE, Mochon E, Boxer LM. Induction of bcl-2 expression by phosphorylated CREB proteins during B-cell activation and rescue from apoptosis. Mol Cell Biol 1996;16:5546–56.

[57] Zhong H, Voll RE, Ghosh S. Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CREBBP/p300. Mol Cell 1998;1:661–71.

[58] Zimmermann N, Acosta AM, Kohlhase J, et al. Confirmation of EP300 gene mutations as a rare cause of Rubinstein-Taybi syndrome. Eur J Hum Genet 2007;15(8):837–42.