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Genetic Heterogeneity in Rubinstein-Taybi Syndrome: Mutations in Both the *CBP* and *EP300* Genes Cause Disease

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CREB-binding protein and p300 function as transcriptional coactivators in the regulation of gene expression through various signal-transduction pathways. Both are potent histone acetyl transferases. A certain level of CREB-binding protein is essential for normal development, since inactivation of one allele causes Rubinstein-Taybi syndrome (RSTS). There is a direct link between loss of acetyl transferase activity and RSTS, which indicates that the disorder is caused by aberrant chromatin regulation. We screened the entire CREB-binding protein gene (*CBP*) for mutations in patients with RSTS by using methods that find point mutations and larger rearrangements. In 92 patients, we were able to identify a total of 36 mutations in *CBP*. By using multiple ligation-dependent probe amplification, we found not only several deletions but also the first reported intragenic duplication in a patient with RSTS. We extended the search for mutations to the *EP300* gene and showed that mutations in *EP300* also cause this disorder. These are the first mutations identified in *EP300* for a congenital disorder.

Introduction

Rubinstein-Taybi syndrome (RSTS [MIM 180849]) is a congenital disorder characterized by mental and growth retardation and a wide range of typical dysmorphic features. Facial dysmorphology includes down-slanted palpebral fissures, a broad nasal bridge, a beaked nose, and micrognathia. Particularly noticeable are the broad thumbs and broad big toes. In addition, patients with RSTS have an increased risk of tumor formation. Although various types of tumors have been described, there is an excess of tumors arising from developmental defects and tumors in brain or neural-crest cell–derived tissue (Miller and Rubinstein 1995). Mutations in the gene encoding the CREB-binding protein (*CREBBP,* also known as "*CBP*"), located on chromosome 16p13.3, were found to be responsible for causing the disorder (Petrij et al. 1995).

The protein, CBP, serves as a transcriptional coactivator (Kwok et al. 1994). It has a transactivation domain but does not specifically bind to DNA. The name of the protein is based on the interaction with the CRE-binding protein (CREB); however, CBP interacts with a large number of other proteins as well. It is thought that CBP acts as an integrator of the signals from various pathways (Goodman and Smolik 2000). Transcription factors downstream from these pathways need to compete with each other for the limited amount of CBP available in the nucleus. The protein forms a physical bridge between the DNA-binding transcription factors and the RNA polymerase II complex. In addition, CBP has intrinsic histone acetyl transferase (HAT) activity (Bannister and Kouzarides 1996). By acetylating histones, CBP opens the chromatin structure at the locus that needs to be expressed, a process essential for gene expression. CBP is also capable of acetylating a large number of other proteins—for example, the transcription factor p53 (Gu and Roeder 1997).

Although RSTS is considered to be an autosomal dominant disorder, patients with RSTS very rarely have children. Almost all mutations, therefore, occur de novo. The mutations found in patients vary from relatively large microdeletions, which remove the entire gene, to point mutations. In addition, five translocations and two inversions disrupting the gene have been reported (Petrij et al. 2000). The microdeletions that remove the entire gene indicate that haploinsufficiency is the ultimate cause of the syndrome. Presumably, at critical moments during development, the amount of CBP drops below a certain threshold because of the loss of

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one allele. How this loss of the allele actually causes the particular symptoms of RSTS, however, is unclear. Nevertheless, we know from studies of patients with missense mutations and splice-site mutations that affect only the HAT domain of CBP that a loss of HAT activity is sufficient to cause the syndrome (Murata et al. 2001; Kalkhoven et al. 2003).

To elucidate the complete spectrum of mutations, we screened 92 patients with RSTS for point mutations, small deletions or insertions, and large deletions and duplications. Because we could not find mutations in the *CBP* gene in the majority of our patients, we assumed that the remaining patients have mutations in other genes.

CBP shares homology with another protein, p300, which is encoded by the *EP300* gene on chromosome 22q13.2 (Lundblad et al. 1995). Both proteins are particularly homologous at their binding sites for transcription factors, and p300 also has a HAT domain. Like CBP, it serves as a transcriptional coactivator. *EP300* is therefore a likely gene candidate to screen, and, indeed, we found three mutations in our analysis of the gene. These are the first mutations found in *EP300* for a congenital disorder, and, in addition, they prove that RSTS is a genetically heterogeneous disorder.

Material and Methods

The majority of DNA samples described in this study were sent to us by clinicians in the Netherlands and many other countries in the form of soluble genomic DNA from patients with a clinical diagnosis of RSTS. DNA from the remainder of the patients was isolated from peripheral blood in our laboratory by use of standard protocols. The study was reviewed by the institutional review board of the Leiden University Medical Center. Parents or guardians of patients provided oral or written consent for molecular studies. DNA from 92 patients was analyzed. The 92 patients included some patients who have been presented in previous publications: mutations affecting the HAT domain have been described by Kalkhoven et al. (2003), and an mRNA deletion in patient 127-2 was described by Petrij et al. (2000), although no genomic mutation was found.

Sequence variants were checked using a set of 98 control chromosomes. When possible, DNA from the parents of patients was analyzed to determine whether sequence variants occurred de novo.

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE was performed with a GC clamp on either the forward or the reverse primer (see table A1 [online only]). To screen the splice sites and branch sites, primers were selected to anneal to the flanking intron sequences and were chosen by use of either WINMELT (Biorad) or MELT-INGENY (Ingeny B.V.) software. All oligonucleotides were synthesized by Sigma-Aldrich. Amplified fragments were analyzed on 9% polyacrylamide gels (acrylamide:bisacrylamide, 37.5:1), with various linear denaturing gradients, optimized for each fragment, on the DCode system from Biorad. Gels were run at 90 V at a constant temperature of 60°C. An acrylamide mixture with 40% formamide and 7 M urea was defined as 100% denaturant, and acrylamide without these denaturing agents was defined as 0% denaturant.

SSCP Analysis

Electrophoresis was performed at room temperature by use of two types of gels. The first type was a polyacrylamide gel (acrylamide:bisacrylamide, 49:1) with 1 × Tris borate EDTA (TBE) without glycerol, and the second type was 0.5 × Mutation Detection Enhancement (National Diagnostics) with 0.6 × TBE and 10% glycerol. During amplification, the fragments for SSCP analysis were radioactively labeled, either by incorporation of α^{32}P-dCTP or by use of primers that were phosphorylated using γ^{32}P-dATP (Amersham). Visualization of the fragments was done using the PhosphorImager (Molecular Dynamics).

Multiple Ligation-Dependent Probe Amplification

Probes were designed for 20 exons of the *CBP* and *EP300* genes. Multiple ligation-dependent probe amplification (MLPA) was performed as described by White et al. (2004); however, for some samples, the amplifications with the multiplex amplifiable probe hybridization (MAPH) and MLPA primers were performed in separate reactions. All samples in which rearrangements were found were tested at least twice.

Sequencing and Restriction Digestions

Sequencing was performed on the ABI 3700 from Applied Biosystems by use of the manufacturer's standard protocol and reagents. Restriction digestions were performed in accordance with the instructions of the manufacturer. Digestions or second sequencing reactions to confirm the first results were done on PCR fragments generated in an independent reaction. The deletion of 8 nt in patient 256-1 was confirmed by PCR with an allele-specific primer, TCCTCCATCTACTAGTAGTG, that skips the deleted part and anneals with two nucleotides after the deletion. The reverse primer has the sequence GTCCTAACCAAATCAAACAG.

Results

Point Mutations and Small Deletions or Insertions in CBP

We screened the entire *CBP* gene for point mutations and small deletions or insertions, primarily by using DGGE, and screened target sequences that were not suited for DGGE by using SSCP analysis. The complete coding sequence and splice sites of the *CBP* gene required a total of 49 fragments, of which 40 were screened using DGGE, which covered ~83% of the coding sequence. Direct sequencing was used to identify the mutation after aberrant bands were found on DGGE or SSCP gels. All mutations were confirmed either by digestion with restriction enzymes, when a restriction-enzyme site was altered, or by a second sequence analysis.

In 92 patients, we found a total of 27 mutations (table 1). The majority is predicted to lead to a premature translation stop, but we also detected five putative missense mutations. Base substitutions leading to a premature stop codon, as well as deletions and insertions leading to frame shifts, can be clearly identified as disease-causing mutations. On the other hand, a change of amino acids is much less clear; however, patients with RSTS, as a rule, have de novo mutations. Since we were able to confirm three of the mutations as de novo, we consider them the most likely to be disease causing. We do not have parental DNA for patients 228-1 and 260-1. All putative missense mutations are at the highly conserved HAT domain of CBP, and the amino acids that are changed have residues that are conserved in both the mouse and the fruit fly (fig. 1).

Unless we have an RNA sample from a patient, we cannot check whether a splice-site mutation actually leads to aberrant splicing. These mutations, however, should also comply with the rule that mutations in patients with RSTS occurred de novo. Except for the mutation in patient 39-1, for which parental DNA was not available, we could confirm the mutations in that way. The G→A mutation in the splice-donor site flanking exon 24 in patient 39-1, however, is at the first position, which—in all splice-donor sites, without exception—should be a guanine. The splice-site mutation in patient 211-3 could be analyzed on RNA isolated from a cell line. Subsequent sequence analysis proved that the mutation in the splice-acceptor site flanking exon 22 leads to a deletion of exon 22 in the processed mRNA (Kalkhoven et al. 2003)

Large Deletions and Duplications in CBP

Previous research suggests that ~10% of the mutations in patients with RSTS are microdeletions affecting the *CBP* gene (Bartsch et al. 1999; Blough et al. 2000). We performed a FISH analysis using five cosmids spanning the entire gene to detect such deletions, when metaphase chromosome spreads of patients were available (Petrij et al. 2000). The recently developed technique of MLPA can also be used to detect microdeletions in soluble genomic DNA (Schouten et al. 2002). Because that was the type of material available to us for the majority of our patients, we performed MLPA to test the *CBP* gene.

The resolution of MLPA is related to the number of probes used. We made a set of 20 MLPA probe pairs, covering most of the *CBP* gene. This allows us to screen for deletions that cannot be detected by FISH. Southern blotting could have been an alternative but is, in our case, impractical—if not impossible—because it requires so much DNA.

The quality of DNA is slightly more critical for MLPA than for a normal PCR; therefore, we could not screen all patients with MLPA who were screened with DGGE and SSCP. In total, we screened 53 patients, and, for controls, we used material from 3 patients with known microdeletions that were detected using FISH, including 1 patient with a deletion of the entire *CBP* gene. Our MLPA analysis detected those positive controls flawlessly, and we found a number of previously undetected mutations. We found a total of nine new deletions, which ranged from single-exon deletions to the deletion of the entire gene. One deletion, that of exon 2, has been described elsewhere for the RNA level (Petrij et al. 2000). At the time, Southern blots did not reveal a deletion in the genomic DNA; therefore, it was not clear whether this was a genomic deletion or a splicing aberration. This mutation has been found in family 127, which consists of an affected mother and child, one of the very few cases of inherited RSTS.

In addition to the nine deletions, we also found a duplication in one individual. Patient 162-1 has a duplication of the first exon of the *CBP* gene. How this leads to the inactivation of this allele is not clear, but a disease-causing duplication of the first exon has been described in Opitz syndrome (Winter et al. 2003).

The exon 1 deletions and duplication were confirmed using extra probe pairs—one probe at the promoter region and three probe pairs in intron 1 (see table A2 [online only]).

Mutations in EP300

Point-mutation detection and MLPA analysis of *CBP* yielded a total of 36 mutations in 92 patients, strongly suggesting that other genes could also be involved in RSTS. The most likely candidate is the *EP300* gene, coding for p300, on chromosome 22q13.1. That gene was screened using the same approach. We used 37 DGGE fragments, which covered ~79% of the coding sequence of *EP300,* and the remaining part was covered

Table 1

List of All Mutations Found in the *CBP* and *EP300* Genes

Gene, Type of Mutation, and Individual	Exon[a]	Mutation[a,b]	Protein Change[c]
CBP:			
Nonsense mutation:			
7-1	Exon 2	c.304 C→T	Q102X
177-1	Exon 5	c.1237 C→T	R413X
212-1[d]	Exon 28	c.4669 C→T	Q1558X
27-1	Exon 29	c.4879 A→T	K1627X
2-1	Exon 31	c.6010 C→T	R2004X
16-1	Exon 31	c.6133 C→T	Q2045X
178-3	Exon 31	c.6283 C→T	Q2095X
Missense mutation:			
209-1[d]	Exon 21	c.3823 G→A[e]	E1278K
201-1	Exon 26	c.4340 C→T[e]	T1447I
260-1	Exon 26	c.4348 T→C	Y1450H
228-1	Exon 27	c.4409 A→G	H1470R
2644[d]	Exon 30	c.4991 G→A[e]	R1664H
Deletion/insertion:			
153-1	Exon 2	c.235 del G	G79fsX86
199-3	Exon 3	c.904_905 del AG[e]	S302fsX348
205-1	Exon 6	c.1381_1388 del 8[e]	G461fsX469
239-1	Exon 6	c.1481 dup A	N494fsX527
203-1	Exon 8	c.1735 dup A[e]	A581fsX586
57-3	Exon 18	c.3396_3400 del 6	P1132fsX1166
10-1	Exon 18	c.3432_3433 del AG	T1144fsX1168
232-1	Exon 21	c.3824 dup T	F1275fsX1282
231-1[d]	Exon 25	c.4256_4258 del CT	S1419fsX1419
34-3	Exon 27	c.4399 del G[e]	V1467fsX1467
213-1[d]	Exon 29	c.4837 del G[e]	V1613fsX1634
Splice-site mutation:			
198-3[d]	Exon 20	c.3779 +5 G→C[e]	
211-3[d]	Exon 22	c.3837 −2 A→T[e]	
47-3	Exon 23	c.3915 −1 G→A[e]	
39-1[d]	Exon 24	c.4133 +1 G→A	
Rearrangement[f]:			
267-1	Del exon 1	c.-198-?_85+? del	
36-3	Del exon 1_2	c.-198-?_798+? del	
74-1	Del exon 1_19	c.-198-?_3698+? del	
15-1	Del exon 1_31	c.-198-?_+1150+? del	
41-3	Del exon 1_31	c.-198-?_+1150+? del	
127-2[g]	Del exon 2	c.86-?_798+? del	
252-1	Del exon 12	c.2159-?_2283+? del	
253-1	Del exon 31	c.5173-?_+1150+? del	
162-1	Dup exon 1	c.-198-?_85+? dup	
EP300:			
254-1	Exon 10	c.1942 C→T[e]	R648X
256-1	Exon 15	c.2877_2884 del 8[e]	S959fsX966
149-1	Del exon 1	c.-1200-?_94+? del[e]	

[a] Del = deletion; dup = duplication.

[b] The mutations are denoted in accordance with the nomenclature published by den Dunnen and Antonarakis (2001). A question mark (?) indicates that the breakpoint is unknown. The changes on the DNA level have been confirmed by restriction digests or by second sequencing reactions. *CBP* mutations are described in relation to GenBank sequence NM_004380, and *EP300* mutations are described in relation to GenBank sequence NM_001429.1, with the A of the ATG start codon counted as nucleotide 1.

[c] The changes on the protein level are predictions.

[d] The mutations in these patients have been published previously by Kalkhoven et al. (2003)

[e] De novo mutation.

[f] Rearrangements found by MLPA.

[g] The mutation found in this patient has been described as an mRNA deletion by Petrij et al. (2000), but the genomic lesion was not found.

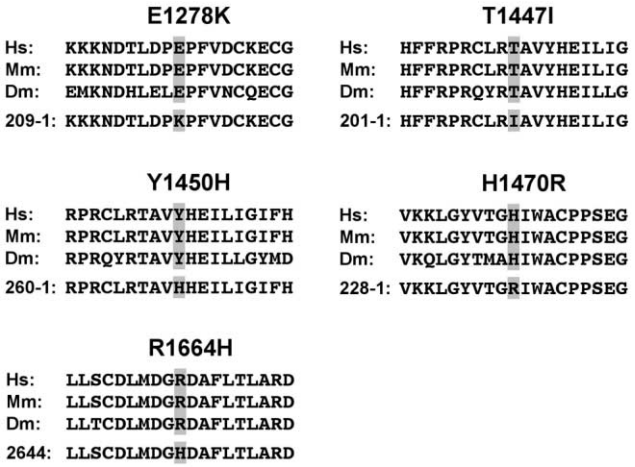

Figure 1 Conservation of amino acids that are predicted to change by missense mutations. All five mutations we found that are predicted to change the amino acid residues are situated in the highly conserved HAT domain. The changed residues are conserved in man (*Homo sapiens* [Hs]), mouse (*Mus musculus* [Mm]), and the fruit fly (*Drosophila melanogaster* [Dm]). Numbers indicate individual patients.

by 10 SSCP fragments. MLPA was performed using a set of 20 exon-specific probe pairs.

Indeed, three inactivating mutations were detected in the *EP300* gene (fig. 2). Two mutations were found using DGGE. One mutation, in exon 10, is a transition (c.1942 C→T) that converts the triplet coding for an arginine at position 648 into a stop codon. The other mutation, in exon 15, is a deletion of 8 nt that predicts a frameshift from codon 959, with a stop codon after 7 aa. The exact location of the 8-bp deletion (c.2877_2884) was confirmed with allele-specific PCR. We analyzed DNA from the healthy parents of both patients by use of DGGE and sequencing and confirmed that the mutations occurred de novo. The biological parentage was confirmed by genotyping with 17 independent markers (data not shown). Both mutations lead to predicted proteins less than half their normal size that do not contain the HAT domain. The third mutation, a deletion of the first exon, was found using MLPA. Four probes revealed this deletion—two probes upstream of exon 1, one in exon 1, and the fourth in intron 1—to be close to the first exon. They all showed a decreased signal, whereas a probe in exon 2 showed a normal dosage (fig. 2*C*). It is probable that this deletion will lead to no expression from the affected allele.

Discussion

We undertook a rigorous screening for point mutations, small deletions or insertions, and large deletions and duplications at the coding section of *CBP* by using genomic DNA from a large set of patients with RSTS. There is neither a predominant type of mutation nor a clear indication of a clustering of mutations within the *CBP* gene. If we take a look at missense mutations, however, we see that they are all situated in the HAT domain of CBP. We have published some of these mutations elsewhere and have shown that they affect the HAT activity of CBP (Kalkhoven et al. 2003). In addition, two articles each reported a de novo missense mutation in the HAT domain, clearly underpinning the importance of this domain in relation to the disorder (Murata et al. 2001; Bartsch et al. 2002). In contrast to our findings, a study by Coupry et al. (2002) reported four putative missense mutations of which only one was situated in the HAT domain. The sequence variations were not found in the other patients, and the affected residues were conserved in mouse and were therefore considered to be causative for RSTS. Whether these mutations actually arose de novo could not be confirmed.

We have found mutations in less than half of the patients (~40%), which is comparable to the outcome of the study by Coupry et al. (2002). DGGE and SSCP analyses, together with detection of nucleotide substitutions, are capable of identifying only relatively small deletions and insertions. To detect larger deletions, we chose to perform MLPA for *CBP* and *EP300*. We now have shown that MLPA is capable of detecting deletions in the *CBP* gene that were previously identified by FISH. Because we have probe pairs corresponding to the majority of exons in both *CBP* and *EP300*, our MLPA screening also negates the need for Southern blotting. The use of MLPA has increased the power to detect mutations, enabling us to find smaller deletions than could be detected using FISH.

Nevertheless, the combined analysis of our samples with both MLPA and DGGE or SSCP analysis found mutations in less than half of the patients. It is probable that some of the patients we screened will be regarded, upon closer clinical examination, as having a different syndrome that resembles RSTS; however, we think the majority of these patients may be considered as having true RSTS. Diagnosis of the syndrome has been performed by many clinicians, but we did not find that some have a significantly better record, in terms of the number of mutations found, than others. In addition, the set of patients with RSTS who were screened by Coupry et al. (2002) had been carefully ascertained, yet mutations were not found in more than half their patients as well. Either the *CBP* gene is mutated at parts that we did not screen, such as the promoter or other regulatory elements, or the mutations are in other genes. The unscreened parts of the *CBP* gene may harbor some mutations, but it is highly unlikely that they contain the majority of RSTS-related mutations that have not yet been identified in these patients. Indeed, RSTS is ge-

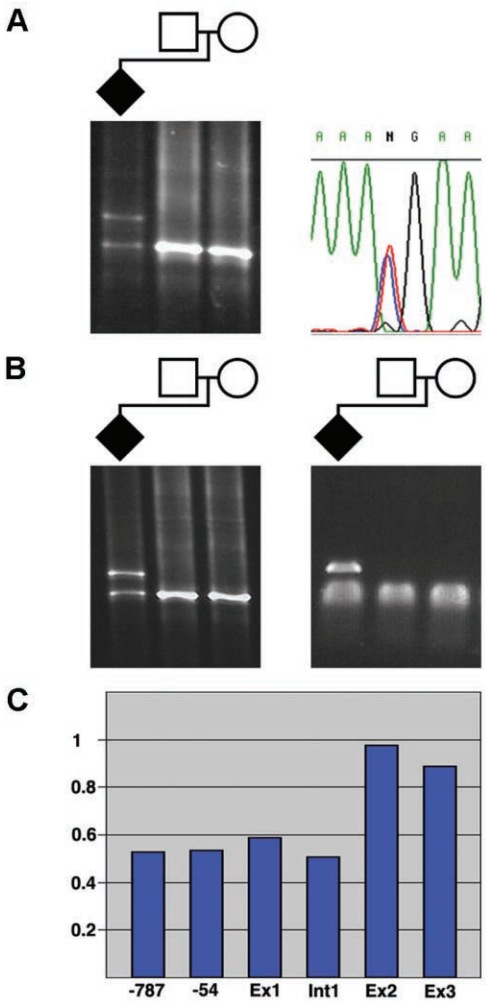

Figure 2 Mutations in *EP300* in patients with RSTS. *A,* Results for patient 254-1. DGGE analysis of patient 254-1 and the healthy parents shows that only the affected child has the mutation. Subsequent sequence analysis revealed a transition, c.1942 C→T, that predicts the protein change p.Arg648X in this patient. *B,* Results for patient 256-1. DGGE analysis of family 256 shows a de novo mutation. The allele-specific PCR confirms the exact location of the deletion seen by sequence analysis. The patient has an 8-bp deletion (c.2877_2884 del) with the following sequence (deleted region is in capital letters; nucleotides of allele-specific forward primer are underlined): gcctcctccatctactagtagCACAGAAGtgaat. The forward primer skips the deleted part and anneals with two nucleotides after the deletion. Only DNA from the patient shows a band of 168 bp, whereas, in the lanes for DNA from the healthy parents, only the prominently visible primer dimers can be seen. *C,* Bar diagram of MLPA results for patient 149-1. MLPA reveals a deletion at the first exon of *EP300*. The *X*-axis shows the DNA probes. The probes upstream of the first exon are at positions 787–716 and 5–54 bp before the transcription start site (indicated by −787 and −54, respectively). Ex = exon; Int = intron. The *Y*-axis represents the dosage of DNA: a dosage of 1 indicates the presence of the normal amount of DNA—that is, both alleles are present—whereas a dosage of ~0.5 typically indicates a deletion of one allele. The diagram clearly shows that the deletion runs from the upstream region of exon 1 into intron 1 and that exons 2 and 3 are present for both alleles. The exact size of the deletion is unknown.

netically heterogeneous, since we identified mutations in the *EP300* gene.

The finding of the *EP300* mutations raises the question of whether there are phenotypic differences associated with *EP300* mutations as compared with *CBP* mutations (fig. 3). The phenotypes of the patients were, in most respects, compatible with classic RSTS: all patients had heavy and arched eyebrows, long eye lashes, a prominent nose with a long hanging columella, and a pouting lower lip. One patient had a small chin. It is interesting that only one patient showed a very mild downward-slanting of the palpebral fissures, and none had the grimacing smile. As mentioned above, all had a shortened and broad thumb and square distal fingertips, and two had visible fetal pads. Also, the big toe was broad in all patients. One patient had a remarkably short first metatarsal bones, giving rise to a very proximal placement of the halluces. Similar dysmorphology has been found in a patient who also has a deleted *CBP* gene (Petrij et al. 1995). In all three patients with *EP300* mutations, the forefoot was broad and the fifth toe was shorter than normal. The small number of patients prevents any of these findings from being firm conclusions.

Strikingly, the number of identified patients with RSTS who have *EP300* mutations, currently three, is small compared with the number of identified patients with RSTS and *CBP* mutations (36 patients). Possibly, this ratio of 1:12 represents the different chances of mutations occurring in these two genes. Alternatively, the *EP300* gene could have a mutation rate equal to that of the *CBP* gene, with the majority of mutation carriers not yet diagnosed as having RSTS. In view of this latter explanation, it is interesting that we found many more polymorphisms in the *EP300* gene, including some that lead to amino acid changes (data not shown). Nevertheless, the majority of point mutations found in the *CBP* gene are likely to lead to truncated proteins, and two mutations in the *EP300* gene are also predicted to truncate the protein, so it is unlikely that the mutation ratio can be entirely explain by differences in the genotype-phenotype relationship. Therefore, we think that the mutation rates of the loci are different.

The *CBP* gene harbors an instable region around exon 2. This region was designated as instable because all translocation and inversion breakpoints could be found in all patients with RSTS, except one—as could all leukemia breakpoints for which CBP functions as a fusion partner. In addition, this same genomic piece of DNA proved very hard to clone at the time of positional cloning of the RSTS gene (Giles et al. 1997). The deletion of exon 2 and the deletions and duplication of exon 1 may be caused by this instable region. The instability in this region, however, cannot explain the majority of deletions found at the *CBP* locus, since most

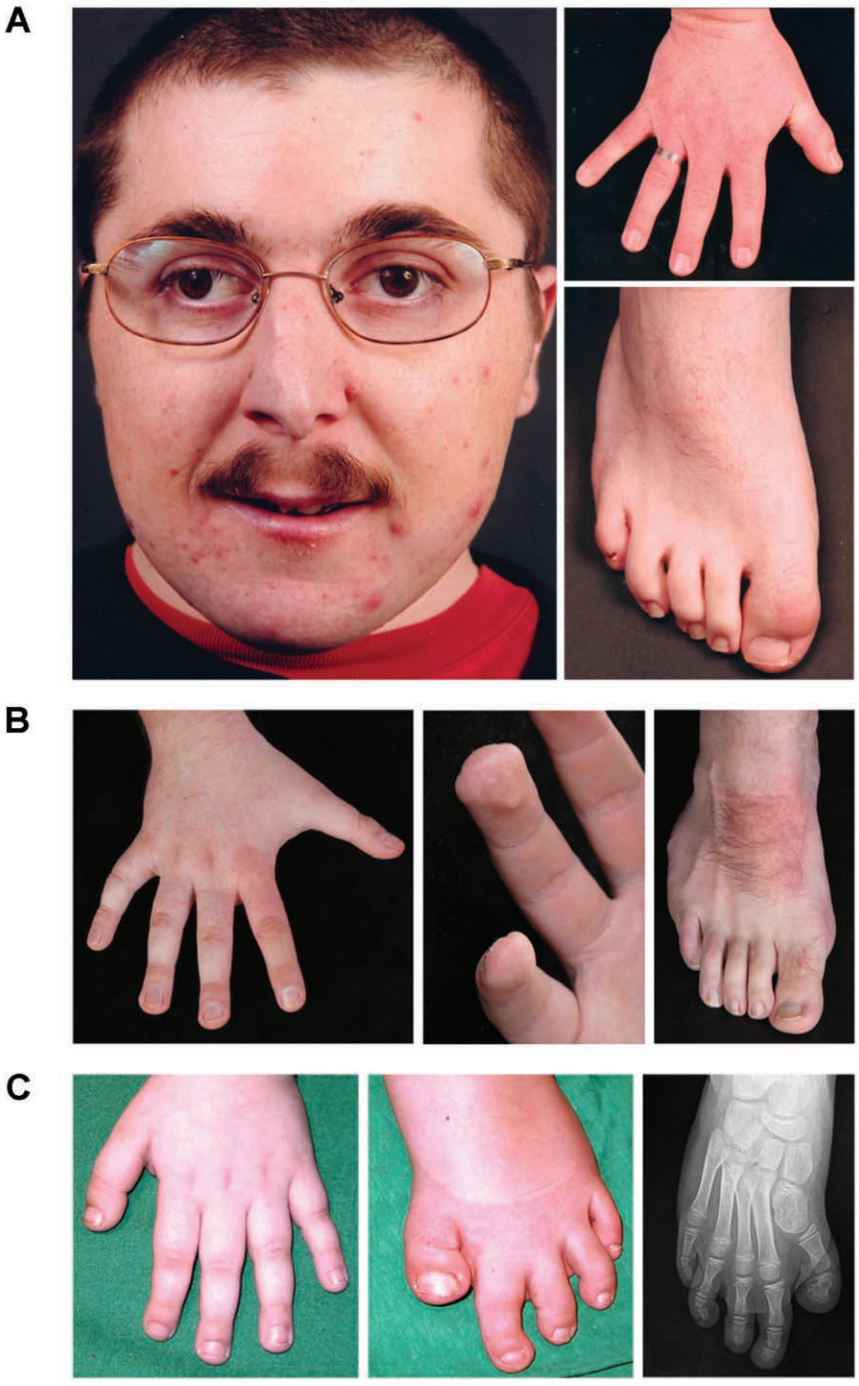

Figure 3 Photographs of the three patients with *EP300* mutations. *A,* The face, a hand, and a foot of patient 149-1. *B,* A hand, a close-up of the fingers with fetal pads, and a foot of patient 254-1. *C,* A hand, a foot, and an X-ray of the foot of patient 256-1. Patient 256-1 has the overall typical appearance of a patient with RSTS, with the exception of the feet. The feet have abnormally short first metatarsal bones, as can be clearly seen in the X-ray photograph. Although this is not a typical feature, it does appear in some other patients with RSTS, as well as in individuals with mutations in *CBP.*

of these deletions have their breakpoints elsewhere (Petrij et al. 2000).

Although CBP and p300 have been shown to have overlapping functions, there are subtle but clear differences between the two proteins (Kalkhoven 2004). During embryogenesis, *CBP* and *EP300* have similar but not completely overlapping expression patterns (Partanen et al. 1999). In addition, experiments with F9 teratocarcinoma cell lines showed that retinoic acid signaling is p300 dependent and does not require CBP, whereas cAMP signaling depends on CBP and not p300 (Kawasaki et al. 1998; Ugai et al. 1999). Recent work with transgenic mice indicated the importance of the acetyl transferase function of p300 in myogenesis, but the acetyl transferase function of Cbp does not seem to be necessary for this process (Roth et al. 2003). The skeletal abnormalities found in heterozygous *Cbp* knockout mice have not been reported for heterozygous *Ep300* knockout mice (Tanaka et al. 1997). We, however, do not find very striking phenotypical differences between patients with mutations in the *EP300* gene versus the *CBP* gene.

Double-heterozygous knockout mice for the *Cbp* and *Ep300* genes resemble the homozygous knockout mice for either gene, in that all three types of mice die in utero, a finding which led to the idea that the combined levels of CBP and p300 are critical during development (Yao et al. 1998). Our findings support this hypothesis and reveal that even a relatively small decrease of either protein has significant developmental consequences. However, it is unclear how a decrease of either protein leads to the specific features of RSTS. Perhaps the partial loss of p300 is compensated for by recruitment of CBP, and subsequent depletion of CBP then leads to RSTS. Alternatively, both proteins could be involved in a common function, and, therefore, the total dosage would be required to prevent a syndrome like RSTS. If so, then this common function has a relationship with the HAT activity of the proteins because loss of only the HAT activity of CBP causes RSTS (Murata et al. 2001; Kalkhoven et al. 2003).

Interestingly, there is a direct link between HAT activity and long-term memory. Heterozygous *Cbp* knockout mice have diminished mental capabilities. Experiments on these knockout mice revealed that inhibiting histone deacetyltransferase could ameliorate the problems with long-term memory seen in the mice (Alarcon et al. 2004). Transgenic mice with a dominant negative *Cbp* gene, in which only the HAT activity was ablated, also showed problems with long-term memory. Again, this could be reversed by a histone deacetylase inhibitor (Korzus et al. 2004). In view of these data, it is possible that other proteins with HAT activity—or with a function coupled to HAT activity—may also be involved in RSTS. After all, the three mutations we have found in the *EP300* gene, together with the *CBP* gene mutations, still leave us with more than half of the cases of RSTS unaccounted for.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for nucleotide sequences [accession numbers NM_004380 and NM_001429.1])

Online Mendelian Inheritence in Man (OMIM), http://www.ncbi.nlm.gov/Omim/ (for RSTS)

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