Abstract  

**Purpose:** Since the identification of **PRKAR1A** mutations in Carney complex, substitutions and small insertions/deletions have been found in ~70% of the patients. To date, no germ-line **PRKAR1A** deletion and/or insertion exceeded a few base pairs (up to 15). Although a few families map to chromosome 2, it is possible that current sequencing techniques do not detect larger gene changes in **PRKAR1A** – mutation-negative individuals with Carney complex.

**Experimental Design:** To screen for gross alterations of the **PRKAR1A** gene, we applied Southern hybridization analysis on 36 unrelated Carney complex patients who did not have small intragenic mutations or large aberrations in **PRKAR1A**, including the probands from two kindreds mapping to chromosome 2.

**Results:** We found large **PRKAR1A** deletions in the germ-line of two patients with Carney complex, both sporadic cases; no changes were identified in the remaining patients, including the two chromosome-2-mapping families. In the first patient, the deletion is expected to lead to decreased **PRKAR1A** mRNA levels but no other effects on the protein; the molecular phenotype is predicted to be **PRKAR1A** haploinsufficiency, consistent with the majority of **PRKAR1A** mutations causing Carney complex. In the second patient, the deletion led to in-frame elimination of exon 3 and the expression of a shorter protein, lacking the primary site for interaction with the catalytic protein kinase A subunit. *In vitro* transfection studies of the mutant **PRKAR1A** showed impaired ability to bind cyclic AMP and activation of the protein kinase A enzyme. The patient bearing this mutation had a more-severe-than-average Carney complex phenotype that included the relatively rare psammomatous melanotic schwannoma.

**Conclusions:** Large **PRKAR1A** deletions may be responsible for Carney complex in patients that do not have **PRKAR1A** gene defects identifiable by sequencing. Preliminary data indicate that these patients may have a different phenotype especially if their defect results in an expressed, abnormal version of the **PRKAR1A** protein.

Carney complex (MIM 160980) is an autosomal dominant multiple endocrine neoplasia syndrome characterized by spotty skin pigmentation, cardiac and other myxomas, and different types of endocrine tumors, including multiple hyperfunctioning adrenal nodules, growth hormone–secreting pituitary tumors, and gonadal and thyroid neoplasias; rarely, malignant psammomatous melanotic schwannomas are present (1–3).

Inactivating mutations in the **PRKAR1A** gene coding for the type 1A regulatory subunit of protein kinase A [PKA; cyclic AMP (cAMP)-dependent protein kinase A enzyme] have been shown to cause the disease (4, 5). To date, 60 different **PRKAR1A** pathogenic mutations have been described; these are mostly single base pair (bp) substitutions and some exonic insertions and deletions that are detectable by PCR-based approaches (5–9). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Note:** A. Horvath and I. Bossis contributed equally to the experimental component of this study and are thus sharing the first authorship. Current address for I. Bossis: University of Maryland School of Veterinary Medicine, College Park, MD 20742.

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To screen for chromosomal abnormalities in Carney complex, fluorescent in situ hybridization analysis has been applied on lymphocyte cell cultures and affected adrenocortical tissues of Carney complex patients (10, 11). A high frequency of somatic PRKAR1A chromosomal alterations is seen in cultured adrenocortical tissues (11); however, no large genomic germ-line rearrangements have been identified (10–12).

Sequencing for PRKAR1A defects has identified germ-line mutations in ~70% of the patients that meet the Carney complex diagnostic criteria (2, 3); in a few families, the disease locus has been mapped to chromosome 2 but chromosome 17 could not be definitely excluded in all of them (2, 3). Thus, the molecular defect of Carney complex remains to be determined in a considerable number of patients, including members of families that map to chromosome 17.

Relatively small intragenic rearrangements involving one or more exons would have been missed by PCR-based strategies, as well as by fluorescent in situ hybridization. We studied 36 unrelated Carney complex patients who did not have small intragenic mutations (by sequencing) and large chromosomal aberrations in PRKAR1A gene (studied by fluorescent in situ hybridization; data not shown); two of the samples were the probands from two unrelated families that have been mapped to chromosome 2 without definite exclusion of the chromosone 17 PRKAR1A locus. All samples were studied by Southern hybridization analysis and other molecular genetic approaches; we identified two large deletions of the PRKAR1A gene in two unrelated patients. This is the first time that PRKAR1A deletions of this size have been identified in patients with Carney complex, a finding with significant implications for the counseling of our patients and for molecular testing of the PRKAR1A gene.

Materials and Methods

Patients. The institutional review boards of National Institute of Child Health and Human Development, NIH, the Mayo Clinic, and Hospital Cochin (Paris, France) approved the contact of the families and the participation of their members in the National Institute of Child Health and Human Development protocols 95-CH-0059 after giving informed consent. Thirty-six unrelated Carney complex patients, who did not have point mutations and large chromosomal aberrations in the PRKAR1A region (negative by fluorescent in situ hybridization; see ref. 11 for probes and methods), were selected for this study. Two of the families were linked to chromosomal locus 2p16, the second region that has been suggested to contain candidate gene(s) for this disease (4). The disease was expressed with a diverse number of manifestations (range, 2-9; mean, 4) and with a variable severity in its expression.

DNA, RNA, and protein extraction. Blood samples were collected from patients, as previously described (5). Lymphocytes were separated from whole blood and transformed by EBV, and the cell cultures were maintained in RPMI 1640 with 1% l-glutamine, 10% fetal bovine serum, and 1% antibiotic/antimycotic agents (13). DNA, RNA, and proteins were extracted from whole blood and cell cultures using standard procedures.

Southern hybridization analysis. For Southern blot analysis, 3 μg of genomic lymphocyte DNA were digested overnight at 37°C with 50 units of EcoRI and KpnI according to the manufacturer’s instructions (New England Biolabs); the digested DNA samples were run on 0.8% agarose gel and transferred onto nylon membrane. The probe for the hybridization was prepared by reverse transcription-PCR amplification of PRKAR1A cDNA spanning exons 2 to 9 and using the following primers: 5′-TAACATTGACGCCGTCCTA-3′ (forward) and 5′-TTCTCAAGCTCCTCCITC-3′ (reverse). The probe was then radioactively labeled by PCR incorporation of [32P]CTP. Hybridization took place overnight at 64°C.

Long-range PCR, cDNA, and protein analysis. Long-range PCR using LA PCR 2.1 kit (TaKaRa) was applied to confirm the deletions observed by Southern blot analysis. Amplification reactions were done under conditions recommended by the manufacturer using the following primers located outside the deleted region: 5′-GATCTTCAAGAGT-GAATCTCTAATCTG-3′ (forward) and 5′-CAAGTAGTACCTGACATATA-GACGTT-3′ (reverse) for the deletion in patient 532.01 and 5′-CAA-GTCAATGATGACTGTTTTGAG (forward) and 5′-CTTAATGCTGAATACGCTC-3′ (reverse). Amplicons from mutation carriers were gel purified and sequenced on an ABI PRISM 3730 automated DNA sequencer according to the manufacturer’s instructions (Applied Biosystems). Sequences were analyzed using Vector NTI Advance (Invitrogen). Protein expression was analyzed by Western blotting.

Generation of PRKAR1A cDNA constructs. Wild-type (wt) PRKAR1A cDNA from a normal adrenal gland and exon 3 skipping PRKAR1A cDNA (PRKAR1A Δ-exon 3) from CAR 564.03 EBV-transformed lymphocytes were amplified using 10× Pfx AccuPrime Reaction Mix, AccuPrime Pfx DNA Polymerase, and the following primers that introduce a Nhel and Kpn1 sites, respectively: 5′-CTTGACTCATG-GAGCTGCGGAGTAGCGGG-3′ (sense) and 5′-GACCGGTCACGGCTC-CAAATTTGGATGTTGAATTAAACG-3′ (antisense). The amplification reaction consisted of 1 min of denaturation at 94°C, 16 cycles of 30 s at 94°C, 30 s at 64°C with temperature decreases of 0.5°C every cycle until touchdown at 56°C, and 2 min at 72°C, 50 cycles at 94°C, 30 s at 56°C, and 2 min at 72°C; and final elongation for 7 min at 68°C. The PCR fragments corresponding to the full-length wt PRKAR1A cDNA and the natural mutant with the exon 3 skipping were gel purified using a MiniElute Gel Extraction kit (Qiagen). These fragments, along with the pcDNA3.1(-) vector (Invitrogen), were double digested with Nhel and Kpn1 and then cloned into the Nhel/Kpn1 sites of the expression vector using T4 DNA ligase and 10× buffer to create PRKAR1A-WT and PRKAR1A Δ-exon 3. The two constructs containing the PRKAR1A open reading frame (ORF) were verified by sequencing before their use in expression studies.

PKA activity. PKA enzymatic activity was measured by a method described previously (13). The assays in a total volume 50 μl were carried out for 15 min at 37°C in the reaction mixture containing 1 mol/L Tris-HCl (pH 7.5), 1 mol/L DTT, 1 mol/L MgCl2, 60 μmol/L L Kempidine (a phosphate acceptor peptide; Leu-Arg-Arg-Ala-Ser-Leu-Gly), 20 μmol/L γ-32P-ATP (25 Ci/mmol), with or without 5 μmol/L cAMP and 100 μmol/L PKA inhibitor (PKI), and 10 μl of the cell extracts. After incubation, the reaction mixtures were spotted onto 0.23-mm phosphocellulose discs and washed thrice in 0.5% phosphoric acid. Filters were air dried and counted by liquid scintillation counter. Total kinase activity represented enzymatic activity after stimulation with cAMP; total PKA-specific activity represented the difference between PKA activity before and after the addition of PKI. PKA-specific activation in response to cAMP reflected the difference between the total PKA-specific activity, as mentioned above, and the baseline activity when neither cAMP nor PKI was present. Statistical analysis of comparisons between groups was undertaken using a two-sample t test; differences were considered significant at P ≤ 0.05. P ≤ 0.10 was interpreted as showing a tendency toward significance.

Twenty micrograms of protein were mixed with 5 μl of 2× SDS protein gel loading solution and 5 μl deionized water, boiled for 5 min, and run on a Novex 10% Tris-glycine gel. Protein was transferred onto a 0.2-μm nitrocellulose membrane. The membrane was blocked with TBS containing 5% nonfat dried milk and 0.5% Tween 20. Proteins were detected with primary antibodies against the main PKA subunits (1:250) and horseradish peroxidase–conjugated secondary antibodies against mouse (1:1,000) or rabbit IgG (1:3,000). All antibodies were diluted in 5% nonfat dried milk in TBS-Tween 20. Bands were detected by enhanced chemiluminescence reagent and densitometer scanning.
Molecular Dynamics. Protein images were quantified with ImageQuant software.

cAMP binding assay. Twenty microliters of the PRKAR1A-WT and PRKAR1A-Δ-exon 3 lysates, prepared as described above, were diluted 10-fold and Tris-EDTA buffer and incubated at 4°C for 1 h with 0.5 μmol/L [8-3H]adenosine 3',5'-cyclic phosphate. The reaction was terminated by the addition of phosphate buffer. The reaction mixtures were then applied via vacuum onto 0.45-μm Millipore filters. Filters were air dried and then counted by liquid scintillation counter. All determinations of cAMP binding were done twice on each sample, normalized by protein content (per μg of total protein), and an average value was calculated for each experiment. To control cAMP binding to other proteins, values were corrected over the amount of PRKAR1A protein in the cell lysates after the transfection, as quantified from the Western blot by densitometry analysis.

Results

Search for large genomic deletions. All samples had been first screened by fluorescent in situ hybridization using the probes and methods described in ref. 11; they were all negative for abnormalities (data not shown). The samples were screened by Southern hybridization analysis after digestion with EcoRI and KpnI. Analysis on EcoRI-digested genomic DNA showed four different PRKAR1A patterns: one corresponding to the wt EcoRI-predicted fragments (28 patients), two unique (each observed in 1 patient), and the fourth shared among 6 patients. All of the observed alterations from the wt pattern were in heterozygote state (Fig. 1A and E).

To assess if the alternative patterns resulted from gene rearrangements or from EcoRI site altering variations, we analyzed the restriction patterns obtained after KpnI digestion. The six DNAs sharing common EcoRI restriction pattern did not show alternative bands after digestion with KpnI; they were considered to have common intronic polymorphic variant that created an EcoRI site. The two DNAs with unique EcoRI restriction pattern expressed additional bands after digestion with KpnI, thus supporting the presence of large gene rearrangements in these patients (Fig. 1B and F). These band patterns were not present in the six control DNA samples from subjects that did not have Carney complex or any related disease. Cumulative analysis of the restriction patterns generated by the two endonucleases suggested the following alterations: (a) deletion of ~4 kb involving the 5’ PRKAR1A region in patient CAR 532.01 and (b) deletion of ~4 kb in the exon 3 area in patient CAR 564.03 (Fig. 1C and G).

To confirm the suggested defects, we did long-range PCR using primers external to the deletions as defined by Southern blot analysis. In the two patients CAR 532.01 and CAR 564.03, shorter products that corresponded to the predicted deletions were seen (1,273 versus 5,149 bp for the wt and 3,068 versus 7,233 bp for the wt, respectively). Lack of the wt allele amplification in these two apparently heterozygous individuals was most likely due to competition with the more advanced shorter products (Fig. 1D and H, respectively).

Fig. 1. Large deletions in the PRKAR1A gene. A and B, Southern hybridization after digestion of the DNA from patient CAR 532.01 with EcoRI and KpnI, respectively. C, schematic representation of the deletion of 3,876 bp in the 5’ PRKAR1A region. Bold, short repeats and palindromic sequences upstream and downstream of the deletion breakpoints; undefined, junction sequence. D, long-distance PCR using primers flanking the suggested breakpoints of the deletion of 3,876 bp in CAR 532.01; only shorter product of 1,273 bp (instead of expected 5,149 bp in the wt DNA) was amplified. E and F, Southern hybridization after digestion of the DNA of patient CAR 564.03 with EcoRI and KpnI, respectively. G, schematic representation of the deletion of 4,165 bp in the region of exon 3 of PRKAR1A. H, long-distance PCR using primers flanking the suggested breakpoints of the deletion of 4,165 bp in CAR 532.01; only the shorter product of 3,068 bp (instead of expected 7,233 bp) was amplified.
Both CAR 532.01 and CAR 564.03 were sporadic cases of the disease: they did not have family history of any related tumors and they, themselves, did not have children. None of the familial cases mapping to chromosome 2 had any PRKAR1A abnormalities.

Breakpoint analysis. To define the boundaries, the long-range PCR aberrant fragments were sequenced by a primer-walking strategy. Breakpoint analysis in patient CAR 532.01 showed loss of 3,876 bp, including part of sequences regulating transcription and exon 1 splicing, without affecting PRKAR1A ORF (see Fig. 1G). This deletion is predicted to depress the expression of the PRKAR1A mRNA, thus leading to an overall decrease of the normal PRKAR1A cellular protein levels. Analysis of the breakpoints in patient CAR 564.03 identified a deletion of 4,165 bp that eliminated exon 3 (see Fig. 1F). Exon 3 is composed of 171 bp and its skipping does not create a frameshift; it was predicted to result in an expressed, in-frame, but shortened PRKAR1A protein.

Analysis of the junctions of the deletion revealed some (although not extensive) homology between sequences adjacent to the deletion end point; some elements that could cause double-strand break were identified upstream and downstream of the newly formed junctions, these including short repeats and palindromic sequences (see Fig. 1C and G, in bold). As with other genes, nonhomologous end-joining mechanisms, involving DNA with minimal homology, may have played a role in these deletions, generating recombination events (14).

Expression on mRNA and protein level. We further analyzed the mRNA and protein expression of the observed variations in patient CAR 564.03, where lipoma tissue and an EBV-transformed lymphocytic cell line were available. Reverse transcription-PCR with primers flanking exon 3 showed the presence of an alternative, shortened mRNA variant (Fig. 2A). Densitometric quantification of the two observed bands showed similar levels of expression (587 densitometric units for the endogenous PRKAR1A, transfected wt PRKAR1A (174.84, 182.27, and 1,752.34 densitometric units for the endogenous PRKAR1A, transfected PRKAR1A-Δ-exon 3 protein content in the transfected cells was similar to the endogenous PRKAR1A, as measured by densitometry of the Western blot bands, and ~10-fold less than the transfect wt PRKAR1A (174.84, 182.27, and 1,752.34 densitometric units for the endogenous PRKAR1A, transfected PRKAR1A-Δ-exon 3, and transfected wt PRKAR1A, respectively; Fig. 3A). We measured basal PKA activity and PKA activity after exposure to cAMP and PKI. Basal PKA activity measurements indicated a ~2-fold increase in PRKAR1A-Δ-exon 3 compared with the wt (77.41 ± 0.02 cpm/mg protein for the wt versus 134.31 ± 0.00 cpm/µg protein for the PRKARI-Δ-exon 3; P = 0.008; Fig. 3B). This difference increased further after exposure to cAMP (189.74 ± 0.05 cpm/µg protein for the wt versus 337.7 ± 0.05 cpm/µg protein for the RI-Δ-exon 3; P = 0.0007). Free kinase activity for both wt and RI-Δ-exon 3 was reduced to similar levels by PKI, an effect that was expected due to its PKA-specific action (66.41 ± 0.02 cpm/µg protein for the wt versus 102.91 ± 0.12 cpm/µg protein for the RI-Δ-exon 3; P = not significant). Thus, PKA-specific activation was significantly higher after transfection with RI-Δ-exon 3 compared with the wt (2.31 ± 0.63–fold; P = 0.05; Fig. 3C).

Increased PKA-specific activation was observed also in EBV-transformed lymphocytes from patient CAR 564.03 compared with wt PRKAR1A lymphocytes (1.31 ± 0.21–fold; P = 0.08; data not shown). Although the elevation of the PKA-specific activity was lower than the one observed in our in vitro experiments, it clearly indicates an in vivo impaired protein function. The relatively milder in vivo effect in lymphocytes is most likely due to the predominantly expressed (under a condition of heterozygosity) wt allele (see Fig. 2C); this is not unexpected because lymphocytes are not affected by Carney complex and the message for the wt PRKAR1A allele remains strongly expressed (13).

We further assessed cAMP binding ability of the exon 3 lacking PRKAR1A in the same transfection experiments that

![Fig. 2](clincancerres.aacrjournals.org) RNA and protein expression studies on PRKAR1A-Δ-exon 3. A, reverse transcription-PCR on cDNA from EBV-transformed lymphocytes from patient CAR564.03: an additional shorter amplicon is seen. B, sequencing of the shortened PRKAR1A cDNA from patient CAR 564.03; the newly formed junction between exons 2 and 4 is shown. C, Western blot analysis on cell lysates from patient CAR 564.03, showing expression of alternative shortened protein.
were used for PKA activity studies. A decrease of the cAMP binding of PRKAR1A-Δ-exon 3 compared with the wt was observed; after the correction for the amount of PRKAR1A protein in the transfected cells, the values were 0.057 ± 0.002 for the wt versus 0.043 ± 0.01 for the PRKAR1A-Δ-exon 3 (P = 0.012; Fig. 3D). Because the wt endogenous PRKAR1A was expressed at similar levels in the PRKAR1A-Δ-exon 3–transfected cells (see Fig. 3A), considerable proportion of the cAMP binding can be contributed to its presence.

**Discussion**

Since the identification of PRKAR1A mutations in Carney complex (4), a large number of sequencing defects of that gene have been found. To date, the reported mutations consist of single base pair substitutions, small deletions, and insertions or combined rearrangements, all of them not exceeding 15 bp (5). We report here for the first time large germ-line PRKAR1A deletions in patients with Carney complex. PRKAR1A is composed of 11 exons, covering a genomic region of ~21 kb and having a coding region of 1,143 bp (starting from exon 2). The first deletion we identified consisted of 3,876 bp, extending into the 5′ region of the gene and eliminating part of the transcription-regulating sequences as well as exon 1. As the starting ATG codon is positioned in exon 2, the deletion did not affect the PRKAR1A ORF and was predicted to lead to decreased mRNA expression. This effect is similar to the one caused by the majority of small PRKAR1A intragenic mutations, which lead to...
premature stop codon generation and subsequent nonsense-mediated mRNA decay. Our experiments with cells bearing these mutations showed elevation of the total cAMP-stimulated kinase activity, consistent with the phenotype of PRKAR1A haploinsufficiency (4, 13, 16). It is unlikely that this deletion represents a rare copy number variation because it was not present in the limited number of control DNA samples that we tested but also because of its correlation with the Carney complex phenotype. The second deletion we found spanned 4,165 bp and eliminated exon 3. Exon 3 is composed of 171 bp and its skipping does not create a frameshift; accordingly, its message escaped nonsense-mediated mRNA decay, a mechanism that we have shown to operate in PRKAR1A mRNAs that contain premature stop codons (5, 15). The shortened message was translated to an “in-frame” expressed exon 3 lacking PRKAR1A isoform, which was shown by Western blot analysis to be present in an ~10-fold decreased abundance (see Fig. 1H). Sequencing of all the coding regions and the promoter of PRKAR1A in this patient showed no other alterations that may lower the expression of the shortened isoforms. A higher rate of protein degradation, caused by instability of the mutant form, may play a role, although it is also possible that our antibody did not have the same affinity with the shorter protein.

PRKAR1A is one of the several types of regulatory subunits that comprise the PKA holoenzyme. Elevation of the cellular cAMP levels leads to activation of the regulatory subunits, dissolution of the holoenzyme, and release of the catalytic subunits. Thus, functionally, inactivation of PRKAR1A is associated with excess PKA signaling in affected tissues (16, 17). The deletion of part of promoter sequences and exon 1 in patient CAR 532.01 is predicted to result in an overall decrease of the normal PRKAR1A message and the total amount of cellular protein, as we have seen in other PRKAR1A mutations leading to stop codons, premature termination of the transcribed sequence, and degradation of the mRNA by nonsense-mediated mRNA decay (15, 18). Thus, his phenotype would not be different from that in other patients with mutations that undergo nonsense-mediated mRNA decay (5, 15); they all share PRKAR1A haploinsufficiency at the protein level. Indeed, patient CAR 532.01 presented with a not uncommon phenotype among patients with Carney complex: that of lentigines and testicular tumors (large-cell calcifying Sertoli cell tumors; Table 1).

The “in-frame” deletion of exon 3 in the second patient (CAR 564.03) led to the expression of a shortened, alternative PRKAR1A protein, lacking 57 amino acids (amino acid positions 60-117), which included the linker sequence between the dimerization/docking and the first cAMP binding domains of PRKAR1A. Exon 3 harbors several amino acid segments that are directly involved in important protein functions, including the primary site for interaction with the catalytic subunit (amino acid 94-98, the inhibitory sequence; refs. 19, 20). Our in vitro experiments on this shortened isoform showed a significant effect of exon 3 skipping mutation on both PKA activity and cAMP binding (see Fig. 3). It is worth mentioning that the observed effect was stronger compared with eight other expressed PRKAR1A mutations, spread over various functional domains of the protein (data not shown). The major effect of the exon 3 deletion on PKA activation is expected to result from the elimination of the primary site for interaction with catalytic subunit and subsequent impaired assembly of the PKA tetramer. Because exon 3 has not been shown to be directly involved in cAMP binding, the observed decreases in cAMP binding capacity were likely due to global changes in the protein conformation that affected most, if not all, of its functions.

Consistent with the above is the severe Carney complex phenotype observed in patient CAR 564.03, both in terms of the number of manifestations and the clinical significance of their expression. The patient was initially diagnosed at age of 32 with psammomatous melanotic schwannomas (21). He subsequently underwent clinical and pathologic evaluation that led to the diagnosis of Carney complex (21, 22). To date, in addition to psammomatous melanotic schwannomas, CAR 564.03 has developed multiple cardiac myxomas (21, 22), blue nevi, spotty pigmentation on the head, neck, and conjunctiva (Fig. 4A), several cutaneous myxomas (Fig. 4B and C), a pituitary tumor, Cushing’s syndrome secondary to the bilateral adrenocortical hyperplasia (primary pigmented nodular adrenocortical disease), and bilateral large-cell calcifying Sertoli cell tumors and thyroid tumors (Table 1). The patient is also abnormally short with large upper/lower segment ratio result-

### Table 1. Phenotype of the two Carney complex patients carrying large deletions in the PRKAR1A gene

<table>
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<tr>
<th>Patient code</th>
<th>Mutation</th>
<th>Carney complex manifestations</th>
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<tbody>
<tr>
<td>CAR 532.01</td>
<td>Deletion of 3,876 bp including exon 1</td>
<td>Lentigineses, LCCSCT</td>
</tr>
<tr>
<td>CAR 564.03</td>
<td>Deletion of 4,165 bp including exon 3</td>
<td>Multiple cardiac myxomas, Blue nevi, Spotty pigmentation on the head, neck, and conjunctiva, Cutaneous myxomas, Lentigineses, PPNAD, Pituitary tumor, LCCSCT, Thyroid nodules, Psammomatous melanotic schwannoma, Growth abnormalities</td>
</tr>
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Abbreviations: LCCSCT, large-cell calcifying Sertoli cell tumors; PPNAD, primary pigmented nodular adrenocortical disease.
figuring from his early puberty and Cushing's syndrome during childhood (Fig. 4D). When considering the number and clinical severity of the manifestations observed in CAR 564.03, it is tempting to speculate that nonsense-mediated mRNA decay actually protects patients with Carney complex from the phenotypic effects of deleterious PRKAR1A mutations, such as those described in patient CAR 564.03.

In conclusion, we have identified PRKAR1A gene deletions in two unrelated Carney complex patients. One of the identified variants leads to in vivo expression of a malfunctioning shortened PRKAR1A protein. Thus, large gene rearrangements may be responsible for Carney complex disease in some patients and appropriate laboratory approaches (Southern blot or exon dosage techniques) may be necessary for their detection; the estimated yield of such a study would be low, however: we found only two deletions among 36 patients with Carney complex or ~6% of PRKAR1A–mutation-negative samples.

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Large Deletions of the *PRKAR1A* Gene in Carney Complex

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