

Harvey Cushing Treated the First Known Patient With Carney Complex

Cynthia J. Tsay,¹ Constantine A. Stratakis,² Fabio Rueda Faucz,² Edra London,² Chaido Stathopoulou,² Michael Allgauer,³ Martha Quezado,³ Terry Dagradi,⁴ Dennis D. Spencer,⁵ and Maya Lodish²

¹Yale University School of Medicine, New Haven, Connecticut 06510; ²Section of Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, Maryland 20892; ³Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland 20892; ⁴Cushing Center, Harvey Cushing/John Hay Whitney Medical Library, Yale University, New Haven, Connecticut 06510; and ⁵Department of Neurosurgery, Yale School of Medicine, New Haven, Connecticut 06519

Context: Carney complex (CNC) is a syndrome characterized by hyperplasia of endocrine organs and may present with clinical features of Cushing syndrome and acromegaly due to functional adrenal and pituitary gland tumors. CNC has been linked to mutations in the regulatory subunit of protein kinase A type I-alpha (*PRKAR1A*) gene.

Design: Tissue samples were taken from the hypothalamus or thalamus or tumors of patients with pituitary adenomas seen and operated on by neurosurgeon Harvey Cushing between 1913 and 1932. Following DNA extraction, sequencing for genes of interest was attempted, including *PRKAR1A*, *AIP*, *USP8*, *GNAS1*, and *GPR101*, to explore the possibility that these mutations associated with acromegaly, CNC, and Cushing syndrome have been conserved over time.

Results: We report a patient described by Dr. Cushing in 1914 with a clinical presentation and postmortem findings suggestive of CNC. Genetic sequencing of the hypothalamus and pituitary adenoma revealed a germline heterozygous p.Arg74His mutation in the *PRKAR1A* gene, a codon previously described as mutated in CNC, but with a novel amino acid change.

Conclusions: This patient is, to our knowledge, the first molecularly confirmed individual with CNC. This case demonstrates the power of modern genetics in studying archived tissues and the importance of recording detailed clinical notes in the diagnosis of disease.

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Freeform/Key Words: acromegaly, archived specimen, Carney complex, Cushing syndrome, genetics, pituitary adenoma

Carney complex (CNC) is a rare syndrome characterized by “myxomas, spotty skin pigmentation, and endocrine overactivity” and first described by J. Aidan Carney in 1985 [1]. Today, CNC is classified as a multiple neoplasia syndrome and shares clinical features with other conditions, such as multiple endocrine neoplasia type 1, McCune-Albright, and Peutz-Jeghers syndromes [2]. Increased endocrine activity in CNC commonly presents with cortisol-producing primary pigmented nodular adrenocortical disease (PPNAD), growth hormone- and prolactin-secreting pituitary adenomas, and large cell calcifying Sertoli cell

Abbreviations: cAMP, cyclic adenosine monophosphate; CNC, Carney complex; CREB, cyclic adenosine monophosphate-response element-binding protein; MAF, minor allele frequency; mt, mutation; pCREB, phosphorylated cyclic adenosine monophosphate-response element-binding protein; PKA, protein kinase A; PPNAD, primary pigmented nodular adrenocortical disease; *PRKAR1A*, protein kinase A type I-alpha; WT, wild-type.

tumors. Therefore, patients with CNC may present with Cushing syndrome, acromegaly, and other endocrine abnormalities. In addition, patients with CNC develop cardiac and skin myxomas and often present with freckles, nevi, and other cutaneous lesions [2]. CNC is inherited in an autosomal dominant manner in most cases, although *de novo* presentation is not infrequent within a family with no known history of the disease [3]. The genes responsible for CNC have been mapped to 2p16 and 17q22-24; mutations in the regulatory subunit of protein kinase A type I-alpha (*PRKARIA*) gene on 17q22-24 cause more than 70% of both inherited and sporadic cases of CNC [4].

From a cohort of 24 patients with pituitary adenomas operated on by neurosurgeon Dr. Harvey Cushing (1869 to 1939) between 1913 and 1932 whose brains and tumors are housed at the Yale School of Medicine, we identified nine with clinical and histological signs of acromegaly using surgical charts and records from the Peter Bent Brigham Hospital and neuropathologist Louise Eisenhardt's (1891 to 1967) "black book." Tissue samples were taken from the hypothalamus, thalamus, or pituitary tumor of relevant patients. Following DNA extraction, sequencing for genes of interest was attempted, including *AIP*, *GNAS1*, *MEN1*, *GPR101*, *USP8*, and *PRKARIA*, to explore the possibility that mutations associated with acromegaly, CNC, and/or Cushing syndrome could be identified. The quality of the DNA was poor in most samples and made the full sequencing of large genes and gene dosage analysis challenging.

Nevertheless, here we report the case of GBS (1879 to 1914), first reported by Dr. Cushing, whose clinical symptoms we now realize fit the diagnostic criteria of CNC. He was also found to carry a novel p.Arg74His mutation in the *PRKARIA* gene, in a codon that was previously described as mutated in CNC [5], but with a different amino acid change.

1. Case Report

GBS was a 34 year-old, married, childless lobster packer and fisherman from Nova Scotia who presented to Dr. Cushing's clinic in Boston in 1913 with a chief complaint of headaches occasionally relieved by vomiting and symptoms including spasms, polyuria, change in disposition, hyperhidrosis, paresthesias of the extremities, and an increase in body size ("I grew all over."). He was referred with a diagnosis of acromegaly, at the age of 20, and complicating diabetes. His family history was notable for "all members of the family on the maternal and paternal side are very large ... none of excessive stature" [6]. Physical examination findings included:

[A] distractable, drowsy and irritable acromegalic of 5 ft. 8 in. in height, weighing 178 lbs. There was considerable adiposity. The skin was smooth, moist, with a few areas of pigmentation and numerous fibromata mollusca scattered over the shoulders and back ... The skeletal changes were pronounced. The thorax for a man of his height was huge and barrel-shaped. The head was massive with extremely marked prognathism ... The X-rays of the skull showed a markedly enlarged and deepened sella turcica measuring 2.5 cm in its anteroposterior direction and 2.2 cm vertically. The frontal sinuses were moderately hypertrophied ... extremities were greatly enlarged. The general physical examination disclosed a greatly enlarged heart with atrial fibrillation ... The local evidences of a pituitary adenoma were curiously inconspicuous aside from the distension of the sella mentioned above ... The fields of vision were normal. The neurological examination in all other respects was negative ... the thyroid was not definitely palpable; both testes were markedly atrophic [6, 7].

Dr. Cushing characterized the intense freckling as "pigmentation fairly well-marked over anterior body with pedunculated, pigmented moles, numerous over chest" [6]. GBS underwent a transsphenoidal operation for sellar decompression, and histology of the biopsied tissue revealed an "eosinophilic adenoma." An experimental bilateral superior cervical sympathectomy was also performed due to the patient's diabetes because "studies

had appeared to show that impulses passed by way of the superior cervical sympathetic to the hypophysis with the resultant discharge of a 'hormone' with glycogenolytic properties" [7,8]. GBS was discharged home but readmitted in 1914 due to edema, cyanosis, dyspnea, and mental disturbances. On physical examination, cardiac hypertrophy, generalized anasarca, and a Cheyne-Stokes rhythm of respiration were noted, and he passed of cardiac failure [7, 9]

Postmortem autopsy findings further pointed toward the possible diagnosis of a multiple neoplasia syndrome, such as CNC, given the extensive hyperplasia and tumors of the endocrine organs. On autopsy, the pathognomonic finding of bilateral "adrenocortical hyperplasia with pigmented nodules" was present, consistent with what we know today as PPNAD. With regards to the adrenals, Dr. Cushing wrote the following in his notes from the pathologist:

Both were equally and symmetrically enlarged; the combined weight was 30 gm; the longest dimension was 8 cm. They were very nodular, firm in consistency, brownish-red in color, and the surface was studded with yellowish areas from 7 to 12 mm in diameter slightly raised above the general surface. Fresh section disclosed well circumscribed and encapsulated nodules apparently composed of mottled cortical tissue.

Histologically the enlargement of the organ proves to be due chiefly to a hyperplasia of the cortex, the medulla being unaffected. The nodular appearance seen on fresh section is shown microscopically to correspond with adenomata of closely packed cortical cells bearing no resemblance to the normal cortical arrangement. The whole tissue is very vascular. A few focal hemorrhages are present. The adenomatous nodules contain a great excess of fat [9].

Other findings included a hypertrophied heart weighing 1000 g with no pathology or signs of atherosclerosis, a hypertrophied liver with central necrosis and congestion, and enlarged kidneys, lungs, and spleen. The presence of an adenoma of the pituitary gland with a large extrasellar extension into the right temporal lobe was noted. Furthermore, there was a colloid goiter with adenomata, acinar adenoma of the pancreas, and adenomas of the parathyroids and thyroid gland along with testicular atrophy and a bilobar hypertrophic thymus (Fig. 1) [7].

2. Materials and Methods

A. DNA Extraction

Multiple brain tissue samples around 500 mg in size were taken from the hypothalamus of the patient, which had been fixed in formalin since his autopsy in 1914 and placed in 10% buffered neutral formalin at room temperature. The brain specimen had been restored in 2009 and placed in fresh formalin at that time. The pH of the fixation solution at the time of restoration was between 4 and 5. Approximately 25 mg of tissue was removed and placed in a 1.5-mL microcentrifuge tube and washed three times for 60 minutes in 1 mL of 1× phosphate-buffered saline (PBS). The tissue was transferred to a VWR Hard Tissue Homogenizing Mix (2.8-mm ceramic beads) (VWR, Radnor, PA), and a solution containing 90 µL of pure-grade water and 90 µL of ZR FFPE DNA MiniPrep 2× Digestion Buffer (Zymo, Irvine, CA) was added. The tissue was disrupted with two cycles of 25 seconds in an OMNI Bead Disruptor 24 (OMNI, Kennesaw, GA). Five microliters of RNase A was added and incubated for 5 minutes at room temperature. From this step onward, DNA was extracted following the ZR FFPE DNA MiniPrep (Zymo, Irvine, CA), using isopropanol to isolate DNA >50 bp and using water at 56°C to elute the DNA in the final step. For the case presented in this patient, separate samples were taken from the hypothalamus and the pituitary tumor itself.

B. Sequencing Analysis of Germline and Pituitary Tumor Samples

For germline DNA derived from the hypothalamus or thalamus of nine patients and in DNA derived from pituitary tumors in three individuals, *AIP*, *MEN1*, *CDKN1B*, and *GPR101* were

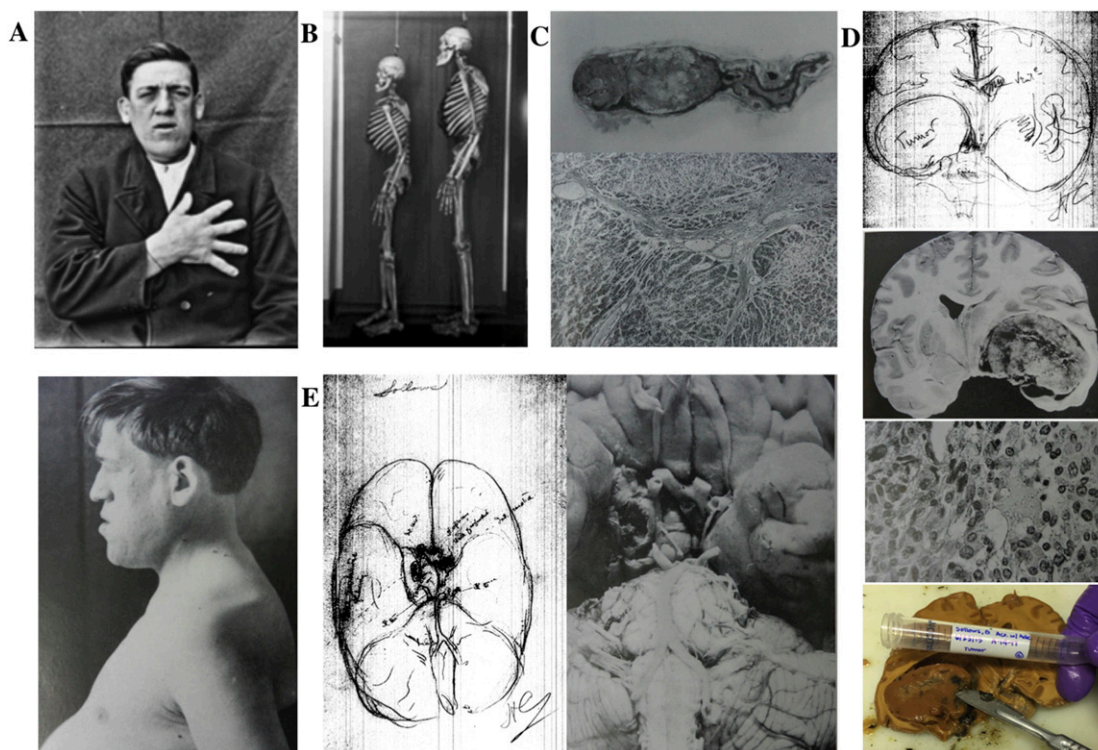


Figure 1. (A) (top to bottom) Photo of GBS demonstrating his enlarged upper extremities in relation to his chest. Profile of patient GBS with clinical signs of growth hormone excess including prognathism and coarse facial features; also evident are skin tags and freckling, the latter we now realize as part of CNC [7]. (B) Skeleton of patient GBS (left) demonstrating enlarged thorax, extremities, and skull compared with another acromegalic patient from the same collection (right) [7]. (C) (top to bottom) Gross specimen of adrenal gland: Scattered throughout are pigmented nodules consistent with PPNAD. A histological slide demonstrates the intersection of four nodules (80 \times) [7] (D) (top to bottom) Original sketch from Dr. Cushing's Brigham notes of the intracranial tumor [6]. Gross specimen of the same pituitary adenoma extending into the right temporal lobe. Histology showed large cells disposed in sheets (600 \times) [7]. Sampling of the same slice of archived brain tissue today, at our laboratory. (E) (left to right) Dr. Cushing's sketch of the inferior brain demonstrating the pituitary adenoma in relation to the optic chiasm [6]. Gross specimen from the autopsy demonstrated that the pituitary adenoma engulfed the optic chiasm [7]. Reproduced courtesy of the Cushing Center, Harvey Cushing/John Hay Whitney Medical Library, Yale University, New Haven, CT.

sequenced as previously described [9, 10]. In tumor samples, additionally *USP8* was sequenced for mutations clustered within the 14-3-3 protein binding motif (codons 718 to 723) as previously described [11]. Pituitary tumor DNA also underwent targeted direct sequencing for the analysis of somatic gain-of-function *GNAS* mutations (codons 201 and 227).

C. *PRKAR1A* Sequencing Analysis

Because a very small amount of highly fragmented DNA was expected, a nested polymerase chain reaction method was used. The complete *PRKAR1A* coding and surrounding intronic sequence was analyzed by classical bidirectional Sanger sequencing, using the internal nested amplicons, applying the primers and conditions described in Supplemental Table 1.

D. *p.Arg74His PRKAR1A* Plasmid

The coding sequence of the human *PRKAR1A* gene (transcript variant 2, NM_212471.2) was cloned into the pSF-CMV-NH2-HA-EKT-*NcoI* plasmid (Oxford Genetics OG93; Oxford Genetics, Oxford, United Kingdom) to express a *N*-terminally HA-tagged protein. The insert was

amplified and cloned within the *NcoI* and *XhoI* restriction sites using the following primers: forward 5'-CATGCCATGGAGTCTGGCAGTACCGC-3', reverse 5'-CCGCTCGAGTCAGACAGACAGTGACACAAAAC-3'. Cloning of the expected sequence was confirmed by Sanger sequencing. The p.Arg74His variant was introduced into the human *PRKARIA* WT template using the QuikChange Lightning site-directed mutagenesis kit (210518-5; Agilent Technologies, Santa Clara, CA), following the manufacturer's protocol. The following mutagenic primers were used: PRKAR1A-R74H_F: CAGAAAGCAGGCACTCATACAGAC-TCAAGGGAG; PRKAR1A-R74H_R: CTCCCTTGAGTCTGTATGAGTGCCTGCTTTCTG.

E. Cell Transfection, Protein Kinase A (PKA) Activity, and cAMP Induction

HEK293 cells grown to 90% confluency in antibiotic-free Dulbecco's modified Eagle medium with 10% fetal bovine serum were seeded in a 12-well plate (120,000 cells/well) 24 hours prior to transfection. Cells were transfected with 0.5 μ g of psF-CMV-NH2-HA-EKT-NcoI plasmid (Oxford Genetics, Oxford, United Kingdom) containing either 1) wild-type (WT) *PRKARIA*, 2) *PRKARIA* with the p.Arg74His mutation (mt), or 3) the empty vector and 4.5 μ L of Lipofectamine 2000 (Life Technologies, Frederick MD) according to the manufacturer's protocol. A nontransfected control group was also included. Transfections were performed in triplicate and the cyclic adenosine monophosphate (cAMP) induction experiment was repeated five times. Forty-eight hours post transfection, the media was removed and replaced with normal growth medium with or without 5 μ M cAMP and incubated at 37°C for 30 minutes. Cells were then harvested in ice-cold 1 \times phosphate-buffered saline with protease inhibitor cocktail set 1 and phosphatase inhibitor cocktail set 1 (EMD Millipore, Billerica, MA). Cells were pelleted at 4°C, and stored at -80°C. Cell pellets were lysed in ice-cold protein extraction buffer [10 mM tris(hydroxymethyl)aminomethane-HCl (pH 7.5), 1 mM EDTA, 1 mM dithiothreitol, 2 mM phenylmethylsulfonyl fluoride (PMSF) and protease inhibitor cocktail I (EMD Millipore, Billerica, MA)] by passage through a syringe fitted with a 22-gauge needle. Lysates were centrifuged at 10,000g for 10 minutes at 4°C. Total protein concentration of supernatant was determined by BCA Assay (Pierce, Rockford, IL). For western blots, 20 μ g of protein was loaded in each well of a Bolt 4% to 12% Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane gel, run at 165 V for 35 minutes, and transferred onto a nitrocellulose membrane at 100 V for 60 minutes. Membranes were probed for phosphorylated cAMP-response element-binding protein (pCREB) (Ser133) (Cell Signaling Technology, Danvers, MA; catalog no. 9198, 1:750) and cAMP-response element-binding protein CREB (Santa Cruz Biotechnology, Dallas, TX; catalog no. sc-240, 1:1000). pCREB and CREB bands were visualized with SuperSignal West Femto and Pico chemiluminescent substrates, respectively (Thermo Fisher, Waltham, MA). Densitometry analysis to determine relative protein expression was performed using Image Laboratory 4.0 software (BioRad, Hercules, CA). PKA enzymatic activity was measured by kemptide assay with P³²-labeled adenosine triphosphate, and data were normalized to account for assay differences in total counts per minute [12]. Data were normally distributed, and thus pairwise *t* tests were used to determine statistical significance between treatment groups. The *P* value cutoff for statistical significance was set at <0.05.

E-1. Role of the funding source

The study sponsors, the National Institute of Diabetes and Digestive and Kidney Diseases and the Eunice Kennedy Shriver National Institute of Child Health and Human Development, did not have a role in the study design, collection, analysis, or interpretation of data.

3. Results

A. Sequencing Analysis of Germline and Pituitary Tumor Samples

Germline tissue was taken from the hypothalamus of eight patients and the thalamus of one patient and sequencing of *AIP*, *MEN1*, *GPR101*, and *CDKN1B* was performed. Of note, there

Table 1. Germline Tissue in Patients With Acromegaly and Pituitary Tumors

Patient No.	Source of Tissue	<i>AIP</i>	<i>PRKAR1A</i>	<i>MEN1</i>	<i>GPR101</i>	<i>CDKN1B</i>
1	Hypothalamus	WT	WT	WT	WT	WT
2	Thalamus	WT	WT	WT	WT	p.Val109Gly
3	Hypothalamus	WT	WT	WT	WT	WT
4	Hypothalamus	n/a	WT	WT	WT	p.Val109Gly
5	Hypothalamus	WT	p.Arg74His	WT	WT	WT
6	Hypothalamus	WT	WT	n/a	n/a	p.Val109Gly
7	Hypothalamus	n/a	WT	WT	WT	WT
8	Hypothalamus	WT	WT	WT	WT	WT
9	Hypothalamus	WT	WT	WT	WT	WT

GBS: patient number 5.

Abbreviations: n/a, sequencing did not work (for all exons or just some of them).

was a p.Val109Gly polymorphism in *CDKN1B* for three patients (Table 1). For the three pituitary tumor samples, there were no mutations found for *AIP*, *MEN1*, *GPR101*, *GNAS1*, *USP8*, or *CDKN1B* (Table 2).

B. *PRKAR1A* Sequencing Analysis

DNA sequencing of brain extract and tumor samples from our patient by Sanger sequencing demonstrated a missense mutation p.Arg74His/c.221G>A in exon 3 of the *PRKAR1A* gene; loss of heterozygosity was present in the pituitary tumor tissue (Fig. 2). Analysis of the GnomAD database [13] showed that the variant p.Arg74His is relatively rare, with a minor allele frequency (MAF) of 0.04%, whereas the p.Arg74Cys variant at the same codon is much more rare with a MAF of 0.0008%. We queried eight of the most commonly used prediction methodologies and found that although a number of tools predicted it to be pathogenic, several others consider it to be tolerated (Table 3).

C. p.Arg74His *PRKAR1A* Plasmid Functional Assay

To investigate the downstream effects of the p.Arg74His mutation, a plasmid bearing the mutant sequence was created and transfected into HEK293 cells. PKA activity was not consistently altered (data not shown), but downstream effects of PKA activity showed significant differences: pCREB (Ser133) and CREB protein levels were quantified by western blot in HEK293 cells, transfected with the WT and the mt *PRKAR1A*, after 30-minute induction with cAMP or nonsupplemented media. The cells bearing the mutation had a higher amount of pCREB after stimulation with cAMP ($P = 0.002$). (Fig. 3). Basal and cAMP-induced pCREB to total CREB ratios were increased by approximately 62% and 35% in cells transfected with 1 μ g of the mutant compared with the WT construct.

4. Discussion

In this study, we showed more than a century after a patient was operated on by Dr. Cushing for acromegaly, that he had a mutation in the *PRKAR1A* gene. The molecular diagnosis of a

Table 2. Pituitary Tumor Samples in Patients With Acromegaly and Pituitary Tumors

Patient No.	Source of Tissue	<i>AIP</i>	<i>PRKAR1A</i>	<i>MEN1</i>	<i>GPR101</i>	<i>GNAS1</i>	<i>USP8</i>	<i>CDKN1B</i>
1	Pituitary tumor	WT	WT	WT	WT	n/a	WT	WT
3	Pituitary tumor	WT	WT	WT	WT	WT	WT	WT
5	Pituitary tumor	WT	p.Arg74His	WT	WT	WT	WT	WT

GBS: patient number 5.

Abbreviations: n/a, sequencing did not work (for all exons or just some of them).

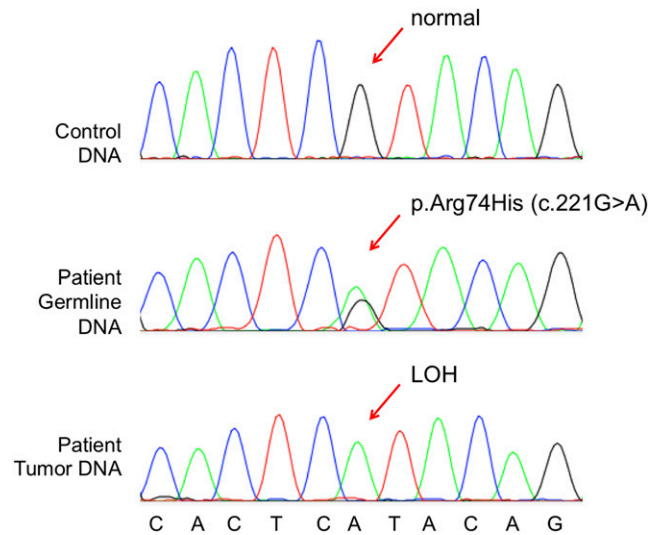


Figure 2. Sanger sequencing of the DNA extracted from tissue preserved in 10% buffered neutral formalin since 1914. A guanine-to-adenine missense mutation, leading to p.Arg74His, on exon 3 of the *PRKAR1A* gene was present in the heterozygous state in germline DNA; loss of heterozygosity is evident in the tumor DNA.

PRKAR1A p.Arg74His mutation, made on DNA successfully isolated from a century-old specimen, combined with the clinical description by Dr. Cushing, and the autopsy findings of pituitary adenoma and pigmented adrenocortical hyperplasia, put together the puzzle pieces of what is now the oldest documented case of CNC. This historic patient has a pathogenic mutation in *PRKAR1A*. While this mutation is relatively rare, a mutation in the same codon which leads to a different amino acid clearly leads to disease. Furthermore, we provide biochemical evidence that this variant has the predicted effect on PKA activity. The finding shows not only the power of modern genetics, but also the significance of clinical phenotyping and recording of an accurate history and physical examination.

This historic case fits the formal diagnostic criteria for CNC as two or more major manifestations are present, including acromegaly due to growth hormone-producing adenoma and PPNAD [22]. Although today most growth hormone-producing tumors in patients with CNC are detected early because of genetic testing, when the disorder was first described, patients presented with large tumors [23]. As reported in 2000, three of the individuals had acromegaly as the primary manifestation of CNC in the context of macroadenomas presenting young at ages 18 to 21 [23]. Invasive, aggressive macroadenoma in one of these individuals eventually led to death from disease in his early 20s.

One may question the pathogenicity of this patient's mutation as the variant p.Arg74His is only relatively rare in the general population, with a MAF of 0.04%, whereas CNC is extremely rare. However, as the genomic databases keep adding thousands of sequencing data

Table 3. Pathogenicity of the p.Arg74His Variant Using Various Prediction Tools

Prediction Methodology	Prediction	Converted Rank Score (Closer to 1 = More Damaging)
MutationTaster [14]	Disease causing	0.81
FATHMM [15]	Damaging	0.95
LRT [16]	Deleterious	0.84
PolyPhen-2 [17]	Probably damaging	0.56
MutationAssessor [18]	Low	0.22
MetaSVM [19]	Tolerated	0.73
Provean [20]	Neutral	0.16
SIFT [21]	Tolerated	0.21

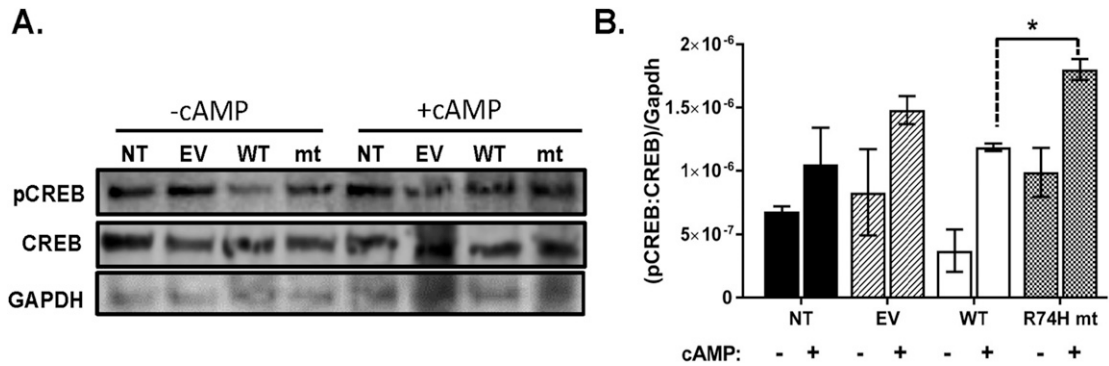


Figure 3. p.Arg74His mutation of *PRKAR1A* alters CREB phosphorylation in basal state and in response to cAMP. The cells bearing the mutation had a higher amount of pCREB than those transfected with WT *PRKAR1A* after stimulation with cAMP (+cAMP) ($P = 0.002$); pCREB levels were not significantly different at baseline (-cAMP) ($P = 0.18$). (A) pCREB (Ser133), CREB, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein levels were quantified by western blot in Hek293 cells after 30-minute induction with 5 μ M cAMP or unsupplemented media. Hek293 cells were first transfected (24 hours) with 1 μ g WT *PRKAR1A*, *PRKAR1A* with R74H mt, or empty vector (EV) plasmid; nontransfected (NT) cells served as an additional control. Transfection experiments were repeated three times with two to three biological replicates per experiment, and western blots for each transfection experiment were repeated twice. (B) Relative pCREB, CREB, and glyceraldehyde 3-phosphate dehydrogenase protein levels were determined by densitometry, and the ratio of phosphorylated to total CREB protein was normalized to the loading control glyceraldehyde 3-phosphate dehydrogenase. Values are mean \pm standard error of the mean. * $P < 0.005$.

daily, it is not unusual to see a clearly pathogenic mutation described; unfortunately one limitation of these databases is that we do not have access to the clinical data associated with these individuals. Another piece of information that supports the likelihood of pathogenicity at the variant found in our historic patient is the high rate of conservation of this amino acid; two bioinformatics sites queried both yielded extremely high predictions for evolutionary conservation at this codon. Using the Genomic Evolutionary Rate Profiling tool measuring the evolutionary conservation of a particular genetic sequence across species [24], the Arg74 codon yielded a score of 5.96 (scores range from -12.3 to +6.17, with the higher score translating to a more evolutionarily conserved codon). Another tool, DANN [25], yielded a score of 0.998 at this site, with the value range from 0 to 1 and scores closer to 1 representing more conserved residues. Finally, somatic loss of the WT allele in the pituitary tumor from this historic patient is another clue that this patient had CNC associated with loss of function of the tumor suppressor *PRKAR1A*.

The p.Arg74His mutation is one of the few *PRKAR1A* mutations that are expressed at the protein level [26]. The effects of the expressed mutations (vs those of the sequence defects leading to *PRKAR1A* haploinsufficiency) are more complex, frequently not altering significantly the PKA enzyme. The p.Arg74His mutation, like the one in the same codon that we described previously [5], had the overall effect of increasing phosphorylation of CREB. Thus, functionally, it had the end effect of increasing cAMP signaling, like all genetic defects causative of CNC.

We conclude that one of Dr. Cushing's patients with acromegaly is the first patient to ever be described with CNC. His diagnosis was molecularly confirmed, showing the significance of good clinical examination and other medical data recording, tissue preservation, and molecular genetics in solving age-old mysteries.

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Correspondence: Maya Lodish, MD, MHSc, 10 Center Drive, Room 2-5142, Bethesda, Maryland 20892. E-mail: lodishma@mail.nih.gov.

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References and Notes

- Carney JA, Gordon H, Carpenter PC, Shenoy BV, Go VL. The complex of myxomas, spotty pigmentation, and endocrine overactivity. *Medicine (Baltimore)*. 1985;**64**(4):270–283.
- Correa R, Salpea P, Stratakis CA. Carney complex: an update. *Eur J Endocrinol*. 2015;**173**(4):M85–M97.
- Stratakis CA, Carney JA, Lin JP, Papanicolaou DA, Karl M, Kastner DL, Pras E, Chrousos GP. Carney complex, a familial multiple neoplasia and lentiginosis syndrome. Analysis of 11 kindreds and linkage to the short arm of chromosome 2. *J Clin Invest*. 1996;**97**(3):699–705.
- Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis CA. Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. *Nat Genet*. 2000;**26**(1):89–92.
- Greene EL, Horvath AD, Nesterova M, Giatzakis C, Bossis I, Stratakis CA. In vitro functional studies of naturally occurring pathogenic PRKAR1A mutations that are not subject to nonsense mRNA decay. *Hum Mutat*. 2008;**29**(5):633–639.
- Cushing HW. Peter Bent Brigham Hospital Surgical Service Notes 1913:21. *Located at: Brain Tumor Registry*.
- Cushing HW, Davidoff LM. *The Pathological Findings in Four Autopsied Cases of Acromegaly With a Discussion of Their Significance*. New York, NY: Rockefeller Institute for Medical Research; 1927.
- Weed L, Cushing H, Jacobson C. Further studies on the role of the hypophysis in the metabolism of carbohydrates. *Bull Johns Hopkins Hosp*. 1913;**25**:40–52.
- Stratakis CA, Tichomirowa MA, Boikos S, Azevedo MF, Lodish M, Martari M, Verma S, Daly AF, Raygada M, Keil MF, Papademetriou J, Drori-Herishanu L, Horvath A, Tsang KM, Nesterova M, Franklin S, Vanbellighen JF, Bours V, Salvatori R, Beckers A. The role of germline AIP, MEN1, PRKAR1A, CDKN1B and CDKN2C mutations in causing pituitary adenomas in a large cohort of children, adolescents, and patients with genetic syndromes. *Clin Genet*. 2010;**78**(5):457–463.
- Trivellini G, Daly AF, Faucz FR, Yuan B, Rostomyan L, Larco DO, Scherthaner-Reiter MH, Szarek E, Leal LF, Caberg JH, Castermans E, Villa C, Dimopoulos A, Chittiboina P, Xekouki P, Shah N, Metzger D, Lysy PA, Ferrante E, Strebkova N, Mazerkina N, Zatelli MC, Lodish M, Horvath A, de Alexandre RB, Manning AD, Levy I, Keil MF, Sierra MdeL, Palmeira L, Coppieters W, Georges M, Naves LA, Jamar M, Bours V, Wu TJ, Choong CS, Bertherat J, Chanson P, Kamenický P, Farrell WE, Barlier A, Quezado M, Bjelobaba I, Stojilkovic SS, Wess J, Costanzi S, Liu P, Lupski JR, Beckers A, Stratakis CA. Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. *N Engl J Med*. 2014;**371**(25):2363–2374.
- Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, Osswald A, Beuschlein F, Meitinger T, Mizuno-Yamasaki E, Kawaguchi K, Saeki Y, Tanaka K, Wieland T, Graf E, Saeger W, Ronchi CL, Allolio B, Buchfelder M, Strom TM, Fassnacht M, Komada M. Mutations in the deubiquitinase gene USP8 cause Cushing's disease. *Nat Genet*. 2015;**47**(1):31–38.
- Nesterova M, Yokozaki H, McDuffie E, Cho-Chung YS. Overexpression of RII beta regulatory subunit of protein kinase A in human colon carcinoma cell induces growth arrest and phenotypic changes that are abolished by site-directed mutation of RII beta. *Eur J Biochem*. 1996;**235**(3):486–494.
- Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;**536**(7616):285–291.
- Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods*. 2014;**11**(4):361–362.

15. Shihab HA, Gough J, Mort M, Cooper DN, Day IN, Gaunt TR. Ranking non-synonymous single nucleotide polymorphisms based on disease concepts. *Hum Genomics*. 2014;**8**:11.
16. Chun S, Fay JC. Identification of deleterious mutations within three human genomes. *Genome Res*. 2009;**19**(9):1553–1561.
17. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;**7**(4):248–249.
18. Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: application to cancer genomics. *Nucleic Acids Res*. 2011;**39**(17):e118.
19. Kim S, Jhong JH, Lee J, Koo JY. Meta-analytic support vector machine for integrating multiple omics data. *BioData Min*. 2017;**10**:2.
20. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid substitutions and indels. *PLoS One*. 2012;**7**(10):e46688.
21. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome Res*. 2001;**11**(5):863–874.
22. Almeida MQ, Stratakis CA. Carney complex and other conditions associated with micronodular adrenal hyperplasias. *Best Pract Res Clin Endocrinol Metab*. 2010;**24**(6):907–914.
23. Pack SD, Kirschner LS, Pak E, Zhuang Z, Carney JA, Stratakis CA. Genetic and histologic studies of somatomammotropic pituitary tumors in patients with the “complex of spotty skin pigmentation, myxomas, endocrine overactivity and schwannomas” (Carney complex). *J Clin Endocrinol Metab*. 2000;**85**(10):3860–3865.
24. Cooper GM, Stone EA, Asimenos G, Green ED, Batzoglu S, Sidow A; NISC Comparative Sequencing Program. Distribution and intensity of constraint in mammalian genomic sequence. *Genome Res*. 2005;**15**(7):901–913.
25. Quang D, Chen Y, Xie X. DANN: a deep learning approach for annotating the pathogenicity of genetic variants. *Bioinformatics*. 2015;**31**(5):761–763.
26. Horvath A, Bossis I, Giatzakis C, Levine E, Weinberg F, Meoli E, Robinson-White A, Siegel J, Soni P, Groussin L, Matyakhina L, Verma S, Remmers E, Nesterova M, Carney JA, Bertherat J, Stratakis CA. Large deletions of the PRKAR1A gene in Carney complex. *Clin Cancer Res*. 2008;**14**(2):388–395.