GNAS mutations in adrenal aldosterone-producing adenomas

Yasuyo Nakajima1), Takashi Okamura1)*, Kazuhiko Horiguchi1), Tamae Gohko1), Tomoko Miyamoto1), Tetsuro Satoh1), Atsushi Ozawa1), Sumiyasu Ishii1), Eijiro Yamada1), Koshi Hashimoto1), Shuichi Okada1), Daisuke Takata2), Jun Horiguchi2) and Masanobu Yamada1)

1) Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi 371-8511, Japan
2) Department of Thoracic and Visceral Organ Surgery, Gunma University Graduate School of Medicine, Maebashi 371-8511, Japan

Abstract. Mutations in GNAS, which encodes Gsα, have been documented in detail, particularly in human pituitary GH-secreting adenomas. Mutations have also recently been reported in adrenal cortisol-producing adenomas (CPAs), in addition to those in the PRKACA gene. However, mutations have not yet been examined in aldosterone-producing adenomas (APAs). Therefore, we herein investigated mutations in the GNAS gene in APAs. Two of the 15 (13%) CPAs with overt Cushing’s syndrome and one of the 9 (11%) CPAs with subclinical Cushing’s syndrome examined had the somatic mutations, p.R201S and p.R201C in the GNAS gene. We identified mutations in the GNAS gene (p.R201C) in 2 out of the 33 (6%) APAs tested, both of which showed autonomous cortisol secretion, while 24 APAs had mutations in the KCNJ5 gene (18 with p.G151R and 6 with p.L168R). These GNAS and KCNJ5 mutations were mutually exclusive in these adenomas. We herein demonstrated for the first time the presence of GNAS mutations in APAs, as well as in some cortisol-secreting adenomas. Our results suggest that these mutations, in addition to mutations in the KCNJ5 gene and other genes such as ATP1A1, ATP2B3 and CACNA1D, may be responsible for the tumorigenesis of APAs and CPAs with subclinical Cushing’s syndrome.

Key words: GNAS, Adrenal adenomas

THE G-PROTEIN-COUPLED RECEPTOR (GPCR) family comprises more than 800 members, which accounts for approximately 4% of encoded human genes and is the largest family of cell-surface receptors involved in signal transduction. GPCRs play crucial roles in various physiological processes including cardiac function, immune responses, neurotransmission, and sensory functions; however, their aberrant activity or expression also contributes to some of the most prevalent human diseases including several tumors [1, 2]. Mutant Ga proteins are known to transform, and mutations in GNAS frequently occur in growth-hormone-secreting pituitary tumors (28%) and thyroid adenomas (5%). Recent sequencing studies using next-generation sequencing reported mutations in GNAS in a wide variety of tumors, including colon cancer, pancreatic tumors, hepatocellular carcinoma, and parathyroid cancer. GNAS mutations have been identified in 4.4% of the 9,486 tumor sequences deposited in the Catalogue of 185 somatic mutations in cancer (COSMIC) database, making it one of the most frequently mutated G proteins in human cancers [2, 3].

Increases in cAMP signaling in the adrenal fasciculata were previously shown to be sufficient to increase cortisol production in adrenal cells [4, 5]. Somatic-activating mutations in GNAS that induce the constitutive activation of the PKA/cAMP pathway have been detected in a subset of adrenal tumors and hyperplasia [6, 7]. Additionally, germline or mosaic mutations in GNAS, PRKAR1A (encoding the regulatory subunit of PKA), and cAMP phosphodiesterases (PDE11A or PDE8B) are also known to enhance cAMP signaling and have been identified in disorders including Cushing’s syndrome and adrenal hyperplasia [4, 8, 9]. Previous studies have demonstrated that mutations in GNAS and PRKACA are mutually exclusive and appear to be associated with small tumors, a young age at presentation, and overt Cushing’s syndrome [4, 10]. Furthermore, other than the PKA pathway, mutations in the CTNNB1

*These two authors contributed equally to this work.

©The Japan Endocrine Society
gene, which encodes β-catenin, and ARMC5 somatic and germline mutations have also been reported in cortisol-producing adenomas and bilateral macronodular adrenal hyperplasia, respectively [4].

We recently reported the high prevalence of somatic mutations in the potassium channel gene, KCNJ5, in more than 70% of Japanese patients with adrenal aldosterone-producing adenomas (APAs) and in some APAs co-secreting cortisol [11-15]. In contrast, the prevalence of these mutations in Western countries was found to be approximately 35-40% in APAs, and cases of APAs co-secreting cortisol have rarely been described [16]. However, GNAS mutations in APAs and APAs co-secreting cortisol have not yet been reported in the literature.

Therefore, we herein investigated GNAS mutations in several adrenocortical adenomas including overt Cushing’s syndrome, subclinical Cushing’s syndrome, APAs, and APAs co-secreting cortisol.

**Subjects and Methods**

**Subjects**

We reviewed the medical records of 15 patients with Cushing’s syndrome, 9 patients with subclinical Cushing’s syndrome, and 33 patients with APAs, who were operated on at Gunma University Hospital between 2007 and 2015. Each subject provided written informed consent, and the study was approved by the Ethics Committee on Human Research of Gunma University. We previously examined mutations in PRKACA genes in 13 patients with Cushing’s syndrome and 8 with subclinical Cushing’s syndrome [17]. In the present study, we added two patients with Cushing’s syndrome and one with subclinical Cushing’s syndrome and then examined mutations in the GNAS gene. Fifteen female cases (aged 53 ± 12 yr (mean ± SD)) were diagnosed with Cushing’s syndrome according to the guidelines published by the Endocrine Society [18]. Primary aldosteronism (PA) was diagnosed as reported previously [11, 19]. We also examined morning and midnight cortisol levels as well as the results of the 1-mg dexamethasone suppression test (DST) in all patients with suspected PA [11, 12, 20]. The failure of cortisol to suppress the level to less than 3.0 μg/dL (139.75 nmol/L) in the DST was used as a parameter for autonomous cortisol secretion in the present study. Cases of McCune Albright syndrome caused by GNAS mutations were not included in this study.

Plasma aldosterone levels were measured with the RIA SPAC-S Aldosterone kit TFB; plasma renin activity, with the RIA Renin IRMA KIT “Daiichi” TFB; plasma cortisol, with the RIA Cortisol kit “TFB” TFB; and ACTH, with ECLIA Eclusys ACTH by Roche Diagnostics.

**RNA extraction and detection of mutations in GNAS cDNA by PCR and direct sequencing**

All adrenal tumor specimens were frozen in liquid nitrogen immediately after removal during surgery. Total RNA was prepared from each adenoma using the RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. cDNA was then reverse-transcribed from 200 ng of total RNA (TaqMan Reverse Transcription Reagents, Applied Biosystems, Tokyo, Japan). In order to sequence GNAS cDNA, 1.0 μL of cDNA was used for the polymerase chain reaction (PCR), as previously reported [17, 21]. PCR solutions were prepared according to the manual for AmpliTaq (Life Technologies, Carlsbad, CA, USA) with a final volume of 50 μL. Each primer used for PCR was set in a different exon, 5'F1, 5'-GATCAAGCAGGCTGACTATGTG-3' (forward primer) and 3'R1, 5'-GATGACCATGTTGAGCTGCTG-3' (reverse primer) in putative exons 7 and 10 (ENSG00000087460). After denaturing at 94°C for 2 min, amplification was performed for 35 cycles at 94°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min using a GeneAmp PCR system 9700 (Applied Biosystems, Tokyo, Japan). PCR products (309 bp) were purified with an AxyPrep PCR clean-up kit (AXYGEN Biosciences, Union City, CA, USA) for sequencing. These samples were directly sequenced using Applied Biosystems 3730xl (Applied Biosystems, Tokyo, Japan).

In cases in which mutations in the GNAS gene was identified, we sequenced the mutated nucleotides of genomic DNA from peripheral blood using the primers 5’-CCAGACCTTTGCTTTAGATTGGC-3’ and 5’-TACTGGCAGTTGACTTTGTCC-3’ under the same PCR conditions as described above. The detection of mutations in the PRKACA and KCNJ5 genes were described previously [14, 17].

**Results**

**Mutations in the GNAS gene in patients with several adrenal adenomas**

We initially examined and confirmed GNAS muta-
GNAS mutations in Japanese adrenal adenomas

In 15 patients with Cushing’s syndrome. All these patients showed symptoms typical of Cushing’s syndrome, and the mean tumor size (maximum diameter) on CT was 28 ± 4 mm. Twenty-four-hour urinary free cortisol excretion in patients, except for one, was more than 200 μg/day, and plasma ACTH levels were undetectable in all patients. Furthermore, the pathologies of all cases were confirmed to be adrenocortical adenomas. We detected mutations in the GNAS gene in 2 out of 15 patients (13%) with Cushing’s syndrome (Fig. 1, 2). The first patient was a 44-year-old female harboring the mutation p.R201C, who had a history of cerebral

Fig. 1  Representative GNAS mutations in cortisol-producing adenomas from patients with Cushing’s syndrome and aldosterone-producing adenomas. Mutations were concentrated at nucleotide C, 601 bp downstream of the translation start site, and the conversion of C to T(A) causing the substitution of arginine (R) by cysteine (C) (or serine (S)) at residue 201 (p.R201C or R201S), and R201 were the most frequently observed mutations in the GNAS gene. Both cases of aldosterone-producing adenomas shown here had mild autonomous cortisol secretion as indicated by 1-mg DST. CS, Cushing’s syndrome; APA, aldosterone-producing adenomas.

Fig. 2  Profiles of GNAS, PRKACA and KCNJ5 mutations in several adrenocortical adenomas. We examined GNAS, PRKACA, and KCNJ5 mutations in 15 patients with Cushing’s syndrome, 9 patients with subclinical Cushing’s syndrome, and 33 patients with aldosterone-producing adenomas. Mutations in the GNAS gene were detected in 2 patients with Cushing’s syndrome (13%), while mutations in the PRKACA gene were noted in 4 (26%). Mutations in the GNAS gene were detected in 1 patient (11%) with subclinical Cushing’s syndrome. Twenty-four patients (72%) harbored KCNJ5 mutations, while 2 (6%) had the GNAS p.R201C mutation in APAs.
infarction, bone fracture, and renal stones. Although she was not obese, she had hypertension and hyperlipidemia. Her tumor was 30 mm in diameter and she had a morning ACTH level of 1 pg/mL, night serum cortisol level of 13.4 μg/dL, 24-h urine cortisol excretion of 318 μg/dL, and 1-mg DST of 13.9 μg/dL. The second patient, who harbored the mutation p.R201S, was a 42-year-old female with a history of osteoporosis, gastric ulcer, obesity, hypertension, hyperlipidemia, and diabetes mellitus. Her tumor was 30 mm in diameter and she had a morning ACTH level of 1 pg/mL, serum cortisol level of 21.7 μg/dL, 24-h urine cortisol excretion of 272 μg/dL, and 1-mg DST of 24.2 μg/dL. Furthermore, we confirmed the absence of a germline mutation in the GNAS gene in DNA from the peripheral blood of the two patients with these mutations. We also examined mutations in the PRKACA gene in the 15 patients with Cushing’s syndrome, and found that 4 patients had somatic mutations in PRKACA (p.L206R). No mutation of PRKACA gene was found in adenomas harboring GNAS mutations (Fig. 2).

We then examined the tumors of 9 patients with no Cushingoid symptoms, but with autonomous cortisol secretion (subclinical Cushing’s syndrome). Serum ACTH levels in most patients were less than 10 pg/mL, and fractionated urine cortisol levels were within normal limits or slightly elevated, but still less than 150 μg/day. None of our patients showed the suppression of serum cortisol levels less than 3 μg/dL with 1-mg DST. The mean tumor size was 31 ± 10 mm. Similar to patients with typical Cushing’s syndrome, we detected the mutation, p.R201C in one of the 9 patients with subclinical Cushing’s syndrome (11%), a 57-year-old female who had a history of myoma uteri with obesity, hypertension, hyperlipidemia, and diabetes mellitus. The tumor was 35 mm in diameter with a morning ACTH level of 8.8 pg/mL, night serum cortisol level of 5 μg/dL, 24-h urine cortisol excretion of 25.2 μg/dL, and 1-mg DST, 3.4 μg/dL. There were no mutations in the PRKACA gene or KCNJ5 gene in these 9 adenomas (Fig. 2).

Patients with APAs frequently have subtle autonomous cortisol secretion. Therefore, we examined 33 APAs, and found that 10 had autonomous cortisol secretion, whereas none of the patients exhibited any Cushingoid signs, except for one who had a slight moon face. Urine cortisol levels in these 10 patients were within the normal range or slightly higher, but were still less than 150 μg/day. However, these 10 patients showed no suppression of serum cortisol levels less than 3 μg/dL with 1-mg DST. Among these patients, 9 showed ACTH<10 pg/mL and/or midnight serum cortisol ≥ 5 μg/dL in addition to 1-mg DST, while one was solely positive in 1-mg DST.

Since we previously reported the high prevalence, of more than 70%, of KCNJ5 mutations, we examined mutations in the KCNJ5 gene and identified 24 APAs that harbored the somatic KCNJ5 mutations of either p.G151R or p.L168R. Six out of 10 APAs with autonomous cortisol secretion had somatic KCNJ5 mutations. We then investigated mutations in the GNAS gene in the 33 APAs and found that 2 harbored the p.R201C mutation. These two APAs did not have KCNJ5 mutations (Fig. 1, 2). The first patient with a GNAS mutation was a 60-year-old female without Cushing’s syndrome, but with morning ACTH < 5pg/mL, a midnight serum cortisol level of 10.5 μg/dL, 24-h urine cortisol excretion of 86.5 μg/dL, and 1-mg DST of 12 μg/dL. She had bilateral adrenal tumors, and 125I adosterol scintigraphy showed bilateral accumulation predominantly in the left adrenal tumor. Adrenal vein sampling suggested predominant aldosterone hypersecretion from the left adrenal tumor. Left adrenalectomy had been performed 7 years previously, and, after that, her morning ACTH level increased to >10 pg/mL, while PAC decreased to 104 pg/mL with an aldosterone/renin activity ratio (ARR) > 200. She was treated with spironolactone and had well-controlled blood pressure. The second case was a 75-year-old man who did not have Cushing’s symptoms, but had a morning ACTH level of 13.8 pg/mL, midnight serum cortisol level of 5 μg/dL, 24-h urine cortisol excretion of 73.3 μg/dL, and 1-mg DST of 3.9 μg/dL. This patient died due to colon cancer 3 years after adrenalectomy. AVS suggested predominant aldosterone hypersecretion from the left adrenal tumor.

**Discussion**

We herein identified, for the first time, the GNAS mutation, p.R201C in 2 out of 33 APAs, both of which showed subtle autonomous cortisol secretion. Furthermore, although many APAs harbored KCNJ5 mutations, KCNJ5 and GNAS mutations were mutually exclusive (Fig. 2). The two patients examined with GNAS mutations met the criteria for PA according to the guidelines of the Japanese and American Endocrine Societies. Although the suppression of morning cortisol with 1-mg DST, which is recommended by the
guidelines of the Endocrine Society, was not observed in these 2 patients, hypercortisonemia was milder than overt Cushing’s disease, reflecting slightly high fractionated urine cortisol and midnight cortisol levels. The recently proposed criteria for subclinical Cushing’s syndrome associated with adrenal incidentaloma by Akehi et al. indicated patients with all conditions including a cortisol level less than 1 mg DST, 1.8 μg/dL, ACTH < 10 pg/mL, and midnight cortisol level ≤ 5 μg/dL for subclinical Cushing’s syndrome [22]. When these conditions were applied to the 2 patients, subclinical Cushing’s syndrome was suspected. It was not possible to determine from pathological findings whether tumors with GNAS mutations produced aldosterone and cortisol; however, it is of interest to note that some APAs possessed GNAS mutations. Although more than 70% of APAs harbor KCNJ5 mutations in Japan, these two APAs with GNAS mutations did not, suggesting that GNAS mutations are also responsible for the tumorigenesis of APA.

APAs co-secreting cortisol have frequently been reported, particularly in Japan [11-13]. We previously showed that the incidence of cardiovascular events was higher in APAs with the autonomous secretion of cortisol than in those without [11]. However, the precise mechanisms underlying the co-secretion of cortisol in APAs remain unclear [22]. We found that approximately 70% of patients with APAs had KCNJ5 mutations, and, although all pure APAs showed mutations, they were less common in APAs secreting cortisol [15].

Regarding cortisol-producing adenomas (CPA), we detected 4 mutations (p.L206R) in the PRKACA gene in CPAs with Cushing’s syndrome, but none in this gene in CPAs with subclinical Cushing’s syndrome, which was consistent with previous findings. Furthermore, we found 2 GNAS mutations (p.R201S and p.R201C) in 15 CPAs with Cushing’s syndrome, and p.R201C in 9 CPAs with subclinical Cushing’s syndrome (Fig. 2). Sato et al. also identified PRKACA mutations in 30 out of 65 CPAs (52.3%) and GNAS mutations (7 p.R201C and 4 p.R201H) in 11 (16.9%) [10]. Thiel et al. detected PRKACA (p.L206R) and GNAS (p.R201C) mutations in 23.1% and 5.8%, respectively, of 52 pure CPAs without autonomous aldosterone secretion [23]. Thiel et al. also examined aldosterone- and cortisol-secreting tumors [23]. They found KCNJ5 mutations in 2 cases of 4 CPAs co-secreting aldosterone, but no mutations in either PRKACA or GNAS genes in any of them.

The difference between GNAS and PRKACA mutations was that the latter were only found in patients with overt Cushing’s syndrome, whereas patients with GNAS mutations showed mild cortisol secretion such as subclinical Cushing’s syndrome or APAs with autonomous cortisol secretion. Therefore, mutations in GNAS may induce mild hypercortisonemia in CPAs and APAs. Mutations in KCNJ5 may be specific for adenomas strongly producing aldosterone, as we previously reported, while those in PRKACA may be specific for tumors showing overt Cushing’s syndrome [17, 23].

After ACTH binds to its receptors, the activation of protein kinase A through the release of the catalytic subunit from the regulatory unit of protein kinase A induces the production of cAMP, which then stimulates the synthesis of cortisol [7]. However, in patients with Cushing’s syndrome, mutations in the PRKACA or GNAS gene induce the constitutive activation of protein kinase A and cortisol secretion [10]. GNAS mutations in CPAs are a subunit of the PKC/cAMP pathway, suggesting a role in cortisol secretion. We detected GNAS mutations in APAs with autonomous cortisol secretion, and GNAS mutations may induce the secretion of cortisol and aldosterone or cortisol alone. GNAS mutations have been identified in many kinds of tumors and cancers, suggesting that these mutations are non-specifically induced in tumors. Further studies are required in order to establish the significance of GNAS mutations in these tumors.

In conclusion, we need to take into account GNAS mutations in APAs without KCNJ5 mutations and also potentially in APAs co-secreting cortisol.

Acknowledgments

We thank all the medical and co-medical staff as well as graduate students involved in patient care. This work was supported by JSPS KAKENHI Grant Numbers 15H04852 (to M.Y.) and 25893026 (to Y.N.), and was partially supported by the Advancing Care of Primary Aldosteronism in Japan Study (to M.Y.) from the Japan Agency for Medical Research and Development, AMED, and the Research on rare and intractable disease, Health and Labour Sciences Research Grants.

Disclosure Summary

All authors have nothing to disclose.
References

1. Pierce JG, Parsons TF (1981) Glycoprotein hormones: structure and function. Annu Rev Biochem 50: 465-495.
2. O’Hayre M, Vázquez-Prado J, Kufareva I, Stawiski EW, Handel TM, et al. (2013) The emerging mutational landscape of G proteins and G-protein-coupled receptors in cancer. Nat Rev Cancer 13: 412-424.
3. Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, et al. (2011) COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res 39: D945-950.
4. Goh G, Scholl UI, Healy JM, Choi M, Prasad ML, et al. (2014) Recurrent activating mutation in PRKACA in cortisol-producing adrenal tumors. Nat Genet 46: 613-617.
5. Simpson ER, Waterman MR (1988) Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. Annu Rev Physiol 50: 427-440.
6. Fragosco MC, Domenice S, Latronico AC, Martin RM, Pereira MA, et al. (2003) Cushing’s syndrome secondary to adrenocorticotropin-independent macronodular adrenocortical hyperplasia due to activating mutations of GNAS1 gene. J Clin Endocrinol Metab 88: 2147-2151.
7. Lefrancois-Martinez AM, Blondet-Trichard A, Binart N, Val P, Chambon C, et al. (2011) Transcriptional control of adrenal steroidogenesis: novel connection between Janus kinase (JAK) 2 protein and protein kinase A (PKA) through stabilization of cAMP response element-binding protein (CREB) transcription factor. J Biol Chem 286: 32976-32985.
8. Bertherat J, Groussin L, Sandrini F, Matyakhina L, Bei T, et al. (2003) Molecular and functional analysis of PRKAR1A and its locus (17q22-24) in sporadic adrenocortical tumors: 17q losses, somatic mutations, and protein kinase A expression and activity. Cancer Res 63: 5308-5319.
9. Almeida MQ, Stratakis CA (2010) Carney complex and other conditions associated with micronodular adrenal hyperplasias. Best Pract Res Clin Endocrinol Metab 24: 907-914.
10. Sato Y, Maekawa S, Ishii R, Sanada M, Morikawa T, et al. (2014) Recurrent somatic mutations underlie corticotropic-independent Cushing’s syndrome. Science 344: 917-920.
11. Nakajima Y, Yamada M, Taguchi R, Satoh T, Hashimoto K, et al. (2011) Cardiovascular complications of patients with aldosteronism associated with autonomous cortisol secretion. J Clin Endocrinol Metab 96: 2512-2518.
12. Adachi J, Hirai Y, Terui K, Nakano T, Fukuda Y, et al. (2003) A report of 7 cases of adrenal tumors secreting both cortisol and aldosterone. Intern Med 42: 714-718.
13. Späth M, Korovkin S, Anke C, Anlauf M, Willenberg HS (2011) Aldosterone- and cortisol-co-secreting adrenal tumors: the lost subtype of primary aldosteronism. Eur J Endocrinol 164: 447-455.
14. Taguchi R, Yamada M, Nakajima Y, Satoh T, Hashimoto K, et al. (2012) Expression and Mutations of KCNJ5 mRNA in Japanese Patients with Aldosterone-Producing Adenomas. J Clin Endocrinol Metab 97: 1311-1319.
15. Yamada M, Nakajima Y, Taguchi R, Okamura T, Ishii S, et al. (2012) KCNJ5 mutations in aldosterone- and cortisol-co-secreting adrenal adenomas. Endocr J 59: 735-741.
16. Mulatero P, Monticone S, Rainey WE, Veglio F, Williams TA (2013) Role of KCNJ5 in familial and sporadic primary aldosteronism. Nat Rev Endocrinol 9: 104-112.
17. Nakajima Y, Okamura T, Gohko T, Satoh T, Hashimoto K, et al. (2014) Somatic mutations of the catalytic subunit of cyclic AMP-dependent protein kinase (PRKACA) gene in Japanese patients with several adrenal adenomas secreting cortisol. Endocr J 61: 825-832.
18. Nieman L, Biller B, Findling J, Newell-Price J, Savage MO, et al. (2008) The diagnosis of Cushing’s syndrome: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 93: 1526-1540.
19. Funder J, Carey R, Fardella C, Gomez-Sanchez CE, Mantero F, et al. (2008) Case Detection, Diagnosis, and Treatment of Patients with Primary Aldosteronism: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 93: 3266-3281.
20. Hiraishi K, Yoshimoto T, Tsuchiya K, Minami I, Doi M, et al. (2011) Clinicopathological features of primary aldosteronism associated with subclinical Cushing’s syndrome. Endocr J 58: 543-551.
21. Ishida E, Yamada M, Horiguchi K, Taguchi R, Ozawa A, et al. (2011) Attenuated expression of menin and p27 (Kip1) in an aggressive case of multiple endocrine neoplasia type 1 (MEN1) associated with an atypical pro-lactinoma and a malignant pancreatic endocrine tumor. Endocr J 58: 287-296.
22. Akehi Y, Kawate H, Murase K, Nagaishi R, Nomiyama T, et al. (2013) Proposed diagnostic criteria for subclinical Cushing’s syndrome associated with adrenal incidentaloma. Endocr J 60: 903-912.
23. Thiel A, Reis AC, Haase M, Goh G, Schott M, et al. (2015) PRKACA mutations in cortisol-producing adenomas and adrenal hyperplasia: a single-center study of 60 cases. Eur J Endocrinol 172: 677-685.