의학석사 학위논문

Protein kinase, cAMP-dependent, alpha catalytic subunit (PRKACA) mutation in adrenal Cushing's syndrome in Korean patients

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2016년 2월

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Protein kinase, cAMP-dependent, alpha catalytic subunit (PRKACA) mutation in adrenal Cushing's syndrome in Korean patients

by
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A thesis submitted to the Department of Medicine
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Professor _____ Chairman

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Abstract

Introduction: Alterations in the cyclic AMP-protein kinase A signaling pathway have been suggested as a cause of autonomous overproduction of cortisol in adrenocortical tumors, resulting in adrenal Cushing's syndrome. Somatic and germline mutations in *PRKACA*, the gene encoding the catalytic subunit alpha of PKA, have been recently identified in 35~65.5% of ACAs associated with overt Cushing's syndrome. The aim of our study was to validate the hotspot mutation Leu206Arg (c.617A→C) in Korean patients, and to analyze the clinical features.

Methods: A retrospective review was conducted on 68 patients who underwent adrenalectomy for overt Cushing's syndrome (CS) from January 2000 to December 2013 at Seoul National University Hospital. We performed *PRKACA* sequencing of DNA from formalin fixed paraffin-embedded (FFPE) blocks of 55 patients of whom specimens were available.

Results: *PRKACA* sequencing for the hotspot mutation Leu206Arg was successful in 48 (87.3%) of 55 DNA samples from FFPE blocks that were available. Leu206Arg *PRKACA* mutation was found in 20 patients (45.5% of adenoma patients). Patients with *PRKACA* mutation had significantly lower levels of DHEA-S compared to patients without *PRKACA* mutation (median 193.0 ng/ml vs. 473.0 ng/ml, p=0.001). Patients with mutated adenomas tended to be older in age (43.9 ± 12.2 vs. 39.5 ± 12.1 years), and were also likely to have more comorbidities such as hypertension and type 2 diabetes. The mean size of adenoma was slightly smaller in patients with *PRKACA* mutation (3.2 ± 0.9 vs. 3.8 ± 1.1cm).

Conclusions: *PRKACA* mutation could be complexly associated with the entire adrenal steroidogenesis pathway. Adrenal Cushing's syndrome patients with this mutation have lower levels of serum DHEA-S; show specific clinical features such as central obesity, buffalo hump, and dry skin; and also seem to have some kind of protection against amenorrhea. In this sample of patients, *PRKACA* mutation was not associated with any difference in biochemical features compared to those without mutation. Further analysis of a larger number of patients is warranted.

Keywords: Cushing′s syndrome, adrenal adenoma, *PRKACA* mutation

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Introduction

The adrenal glands are endocrine organs located above the kidneys. They consist of two layers – the outer cortex, and the inner medulla – that produce different hormones that are essential in various biological functions. Tumors, infections and genetic mutations may impair the normal function of the adrenal glands, resulting in the dysregulation of adrenal hormone production. Adrenal incidentalomas are adrenal masses that are otherwise unsuspected, discovered upon radiologic imaging. The incidence of adrenal incidentalomas has been reported to be as high as 5% of the population, up to 8.7% in autopsy series (15). Adrenal masses are classified either as malignant or benign, and functioning or non-functioning. Among incidentalomas, 80% are nonfunctioning adenomas, 5% are the cause of subclinical Cushing's syndrome, 5% are pheochromocytoma, 1% cause hyperaldosteronism, and about 5% are adrenocortical carcinomas. Malignant and functioning tumors can be treated with surgical resection.

Adrenal Cushing's syndrome results from an excess of cortisol production by adrenocortical tumors (ACT), leading to typical symptoms such as moon face, buffalo hump, central obesity, and abdominal striae (4,5). It is associated with increased morbidity and mortality, especially when remained undiagnosed or untreated (16). Cushing's syndrome is generally classified into ACTH (adrenocorticotropic hormone)-dependent and ACTH-independent Cushing's syndrome. ACTH-independent Cushing's syndrome accounts for about 20% of cases, which includes adrenal adenoma, adrenal carcinoma,

macro- and micro-nodular adrenal hyperplasia (5).

Several important molecular pathways, such as the Wnt signaling pathway, and the cyclic AMP (cAMP)-protein kinase A (PKA) signaling pathway, have been linked to the formation of ACTs (17). Alterations in the cAMP-PKA signaling pathway have been suggested in playing a role in the pathogenesis of autonomous overproduction of cortisol from these tumors (7). Mutations in the genes encoding guanine nucleotide binding protein (GNAS1), armadillo repeat containing 5 (ARMC5), phosphodiesterase 11A (PDE11A), phosphodiesterase 8B (PDE8B), and the regulatory subunit I alpha of PKA (PRKAR1A), resulting in PKA activation, have been reported in association with adrenocortical adenomas (ACA), and hyperplasias of the adrenal cortex (8-10).

Somatic and germline mutations in *PRKACA*, the gene encoding the catalytic subunit alpha of PKA, have been recently identified in 35~65.5% of ACAs associated with overt Cushing's syndrome (1-3, 11). *PRKACA* mutations have been observed in younger patients, with smaller adenomas, and more overt features compared to patients without *PRKACA* mutations (1,2,12). Meanwhile, Cao et al. only detected differences in gender, with a female preponderance in ACT patients with the hotspot somatic L205R mutation in *PRKACA* (3).

The aim of our study was to validate the hotspot mutation Leu206Arg (c.617A→C) and to analyze the clinical features of patients with *PRKACA* mutation in Korean patients.

Materials and Methods

A retrospective review was conducted on 68 patients who underwent adrenalectomy for overt Cushing's syndrome (CS) from January 2000 to December 2013 at Seoul National University Hospital. We performed *PRKACA* sequencing of DNA from formalin fixed paraffin-embedded (FFPE) blocks of 55 patients of whom specimens were available in the archives of the Department of Pathology. The study conducted in accordance with the guidelines proposed in The Declaration of Helsinki involving humans, and was approved by the ethics committee of Seoul National University Hospital.

The diagnosis of adrenal Cushing's syndrome was made when patients presented with typical features and biochemical results of hypercortisolism. Patients were screened with either the 24-hour urine free cortisol (UFC), overnight dexamethasone suppression test (DST) or the late-night salivary cortisol test (4). ACTH levels were measured to determine the cause of excess cortisol. All patients suspected of having adrenal lesions underwent a computed tomography (CT) scan. Unilateral or bilateral adrenalectomy was performed either with the open method or laparoscopically. In all cases, pathology confirmed the diagnosis.

DNA extraction and *PRKACA* sequencing

An experienced pathologist specializing in adrenal tumors reviewed all histological sections of ACTs obtained from patients with adrenal Cushing's syndrome, and FFPE blocks containing the most tumor tissues were selected.

DNA was extracted from 55 paraffin embedded blocks of ACTs. Tumor DNA was extracted by using the QIAamp DNA FFPE tissue kit (Qiagen), and was evaluated for quantity and quality by spectrophotometry at 260nm. DNA purification was performed using the HiYield Gel/PCR DNA Fragments Extraction Kit (Real Genomics) when necessary. Custom TaqMan SNP assay (TaqMan) for the hotspot mutation Leu206Arg was used for PCR, with the following primers:

forward, 5'-CTTGTTGTAGCCCTGGAGCA-3';

reverse, 5'- CCACAGGTGACAGACTTCGG-3'.

Direct bidirectional sequencing was performed with ABI 3130XL Genetic Analyzer and BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA).

Statistical analysis

SPSS version 22 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. Continuous data were compared using Student's t-test or Mann-Whitney U test where appropriate. The nominal data were analyzed with chi-square test or Fisher exact test. A p-value <0.05 defined statistical significance. Data are expressed as mean ± standard deviation, median [interquartile range], or number (%).

Results

Between January 2000 and December 2013, 68 patients were treated for adrenal Cushing's syndrome with overt clinical and biochemical features. FFPE blocks were available for 55 patients. The mean patient age was 41.5 ± 12.0 years (range, 21.1 – 66.0 years); 45 (81.8%) were female and 10 were male. Clinical characteristics of the patients are summarized in Table 1. Symptoms and signs patients most commonly presented with were central obesity (n=33, 60.0%) and moon face (n=33, 60.0%), followed by easy bruisability (n=21, 38.2%), amenorrhea (n=17, 37.8% females), and buffalo humps (n=19, 34.5%). The mean tumor size of 50 adenomas was 3.5 ± 0.9cm. Mean serum cortisol was 19.2 ± 7.5 ug/dl, median urinary free cortisol was 376.4 [255.0 – 976.0] ug/24h.

PRKACA sequencing for the hotspot mutation Leu206Arg was successful in 48 (87.3%) of 55 DNA samples from FFPE blocks that were available. Among these 48 patients, 44 were unilateral (26 left, 18 right) and four were bilateral. Pathology revealed adenomas in all unilateral adrenalectomy patients (one with two adenomas). Three of the bilateral patients were diagnosed with adrenal hyperplasia, and one was found with no diagnostic abnormality in pathology (Fig. 1). Leu206Arg *PRKACA* mutation was found in 20 patients (41.7% of entire cohort, 45.5% of adenoma patients) (Fig. 2).

Table 1 Characteristics of patients surgically treated for adrenal Cushing's syndrome (n = 55)

	Mean ± SD
General Characteristics	
Age (yrs)	41.5 ± 12.0
BMI (kg/m^2)	24.6 ± 4.8
Female (n, %)	45 (81.8%)
Comorbidities (n, %)	
Hypertension	34 (61.8%)
Diabetes	14 (25.5%)
Signs and symptoms (n, %)	
Central obesity	33 (60.0%)
Moon face	33 (60.0%)
Easy bruisability	21 (38.2%)
Amenorrhea	17/45 (37.8%)
Buffalo humps	19 (34.5%)
Osteoporosis	12 (21.8%)
Oily skin	7 (12.7%)
Dry skin	4 (7.3%)
Edema	17 (30.9%)
Abdominal striae	8 (14.5%)
Hirsutism	11 (20.0%)
Muscle weakness	4 (7.3%)
Psychological symptoms	2 (3.6%)

Table 1 Continued

	Mean ± SD
Hormonal parameters	
ACTH (pg/ml)	14 [10.0-24.0] (n=54)
Cortisol (ug/dl)	19.2 ± 7.5 (n=54)
Urinary free cortisol (ug/24h)	376.4 [255.0-976.0] (n=52)
DHEA-S (ng/ml)	304.0 [193.0-625.0] (n=41)
Urine VMA (mg/24h)	2.8 ± 1.2 (n=41)
Adenoma size (cm)	3.5 ± 0.9 (n=50)
Laparoscopic surgery (n, %)	48 (87.3%)

Figure 1 Overview of the cohort – pathologic diagnosis according to mutation

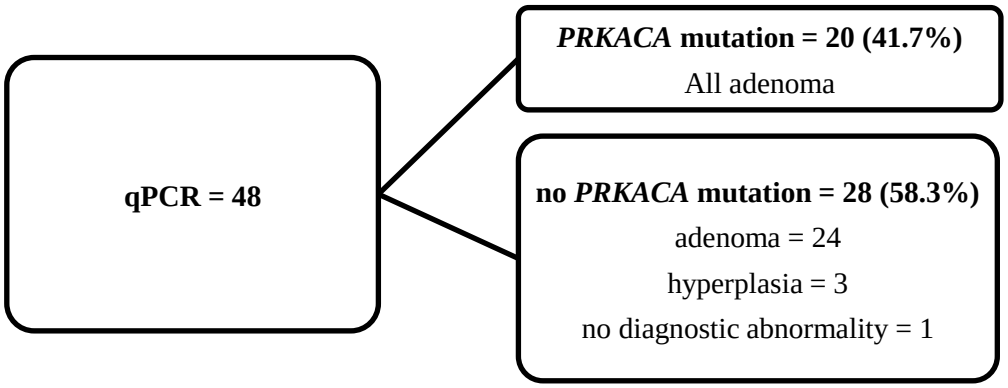
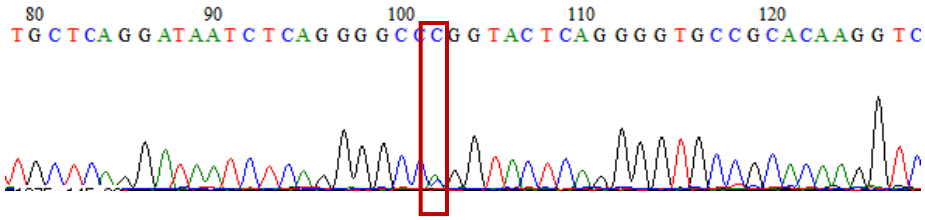
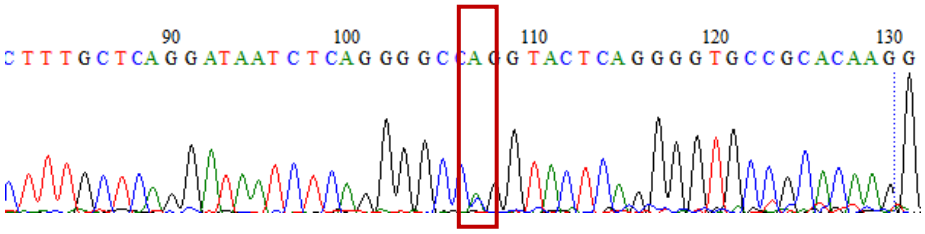


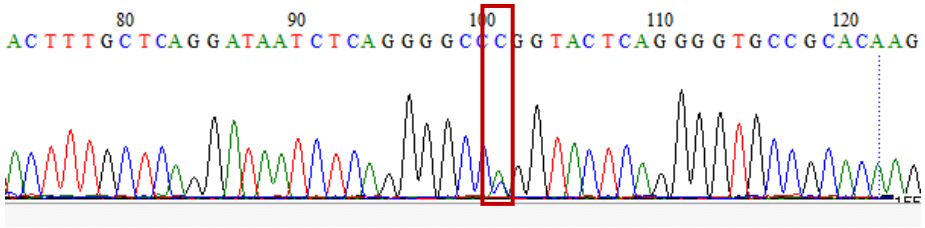
Figure 2 Validation of somatic mutation in hotspot region Leu206Arg of *PRKACA* gene – PCR and Sanger sequencing were performed in tumor from patients with ACTs



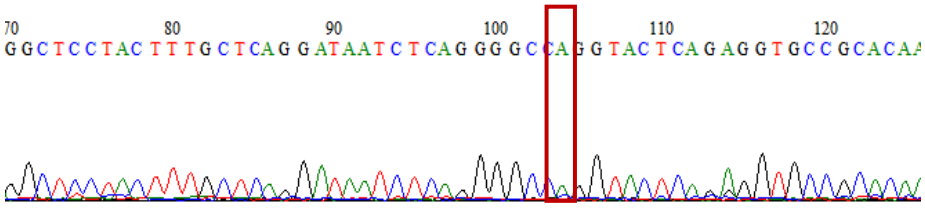
ACT40



ACT45



ACT54



ACT55

Typical features that were commonly seen in this cohort, mentioned above, were compared between patients with *PRKACA* mutation and those without mutation (Table 2). 17.6% patients with *PRKACA* mutation complained of amenorrhea, compared to 54.5% patients without mutation (p=0.019). More patients with *PRKACA* mutation tended to present with central obesity (70.0% vs. 57.1%, p=0.364) and a buffalo hump (45.0% vs. 28.6%, p=0.241); weight gain was similar between both groups (45.0% vs. 42.9%, p=0.883). With regard to skin problems, 15.0% patients with *PRKACA* mutation had dry or brittle skin, while none of the patients without mutation had brittle skin (p=0.066); more patients without the mutation had oily skin (17.9% vs. 5.0%, p=0.379). Otherwise, there were no differences between both groups in moon face, bruisability, and hirsutism.

Biochemical results of patients with *PRKACA* mutation and those without the mutation are summarized in Table 3. There were no statistical differences in ACTH, cortisol or urinary free cortisol levels between both groups. They also did not differ in other biochemical measurements after low dose dexamethasone suppression test (LDDST) and high dose dexamethasone suppression test (HDDST). The only difference between both groups was shown in DHEA-S (dehydroepiandrosterone sulfate) levels. Patients with *PRKACA* mutation had significantly lower levels of DHEA-S compared to patients without *PRKACA* mutation (median 193.0 ng/ml vs. 473.0 ng/ml, p=0.001) (Fig. 3)

After excluding patients with bilateral lesions, we analyzed only adenoma patients for clinical and hormonal features according to *PRKACA* mutation status (Table 4). Patients with *PRKACA* mutated adenomas tended to be older

in age (43.9 ± 12.2 vs. 39.5 ± 12.1 years), and were also likely to have more comorbidities such as hypertension and type 2 diabetes. The median size of adenoma was slightly smaller in patients with *PRKACA* mutation (3.0 vs. 3.5cm, p=0.061). There were no significant differences in serum cortisol, urinary free cortisol and serum ACTH between adenoma patients with and without *PRKACA* mutation.

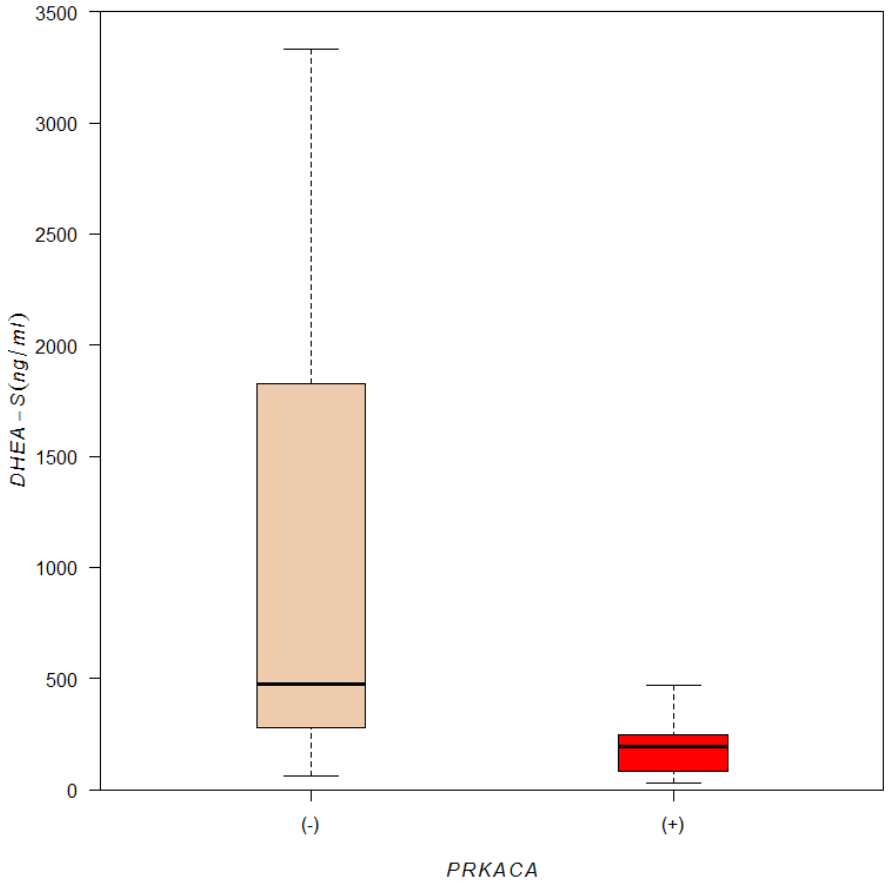
Table 2 Signs and symptoms of patients with *PRKACA* mutation vs. no mutation

	PRKACA mutation (n=20)	No *PRKACA* mutation (n=28)	*p*-value
Signs and Symptoms			
Cushinoid appearance	14 (70.0%)	24 (85.7%)	0.282
Incidentaloma	11 (55.0%)	9 (32.1%)	0.113
Central obesity	14 (70.0%)	16 (57.1%)	0.364
Weight gain	9 (45.0%)	12 (42.9%)	0.883
Plethora	0 (0%)	2 (7.1%)	0.504
Moon face	12 (60.0%)	17 (60.7%)	0.960
Buffalo hump	9 (45.0%)	8 (28.6%)	0.241
Abdominal striae	2 (10.0%)	6 (21.4%)	0.440
Petechiae	0 (0%)	1 (3.6%)	>0.999
Dry skin	3 (15.0%)	0 (0%)	0.066
Oily skin	1 (5.0%)	5 (17.9%)	0.379
Edema	4 (20.0%)	9 (32.1%)	0.351
Easy Bruisability	7 (35.0%)	10 (35.7%)	0.959
Muscle weakness	0 (0%)	3 (10.7%)	0.255
Amenorrhea	3/17 (17.6%)	12/22 (54.5%)	0.019
Hair loss	2 (10.0%)	2 (7.1%)	>0.999
Hirsutism	3 (15.0%)	6 (21.4%)	0.716
Psychological symptoms	0 (0%)	2 (7.1%)	0.505

Table 3 Biochemical results of patients with *PRKACA* mutation vs. no mutation

	PRKACA mutation (n=20)	no *PRKACA* mutation (n=28)	*p*-value
Biochemical results			
ACTH (pg/ml)	13.0 [10.0-26.3] (n=20)	14.0 [10.0-26.0] (n=27)	0.735
Renin (ng/ml/hr)	1.5 [1.0-5.4] (n=14)	1.1 [0.7-2.5] (n=16)	0.417
Aldosterone (pg/ml)	49.3[15.4-99.8] (n=16)	26.0[11.4-41.4] (n=17)	0.126
Cortisol (ug/dl)	17.5 ± 4.9 (n=20)	19.4 ± 9.3 (n=27)	0.369
DHEA-S (ng/ml)	193.0 [82.5-244.5] (n=11)	473.0 [279.0-1824.0] (n=25)	0.001
LDDST Cortisol (ug/dl)	15.0 [13.2-20.8] (n=16)	18.6 [13.2-22.7] (n=20)	0.373
LDDST UFC (ug/24h)	267.5 [160.6-769.0] (n=16)	350.5 [238.5-865.3] (n=20)	0.324
HDDST Cortisol	18.0 ± 4.6 (n=14)	20.7 ± 7.5 (n=21)	0.240
HDDST UFC(ug/24h)	421.0 [215.8-794.5] (n=15)	586.0 [237.0-872.0] (n=22)	0.412
Urine VMA (mg/24h)	2.8 ± 1.3 (n=18)	2.8 ± 1.2 (n=19)	0.876
Urine Metanephrine (ug/24h)	1.0 [0.1-41.9] (n=18)	25.5 [0.1-55.8] (n=21)	0.554
UFC (ug/24h)	328 [262-698] (n=19)	436.[236.8-995.6] (n=27)	0.540
Na (mmol/l)	143.1 ± 2.2	142.2 ± 2.6	0.228
K (mmol/l)	4.1 ± 0.4	4.0 ± 0.5	0.500
Cl (mmol/l)	105.1 ± 3.0	104.0 ± 3.6	0.272

Figure 3 Box plot illustrating the difference of serum DHEA-S level



* Outliers were omitted. Values are median (line), interquartile range (box), and range (whiskers).

Table 4 Clinical features and biochemical results of adenoma patients with *PRKACA* mutation vs. no mutation

	PRKACA mutation (n = 20)	no *PRKACA* mutation (n = 24)	p-value
General Characteristics			
Age at operation (yrs)	43.9 ± 12.2	39.5 ± 12.1	0.240
Gender (n, %)			>0.999
Male	3 (15%)	3 (12.5%)	
Female	17 (85%)	21 (87.5%)	
Comorbidities			
Hypertension (n, %)	15 (75%)	13 (54.2%)	0.153
Diabetes (n, %)	6 (30%)	3 (12.5%)	0.261
Biochemical markers			
ACTH (pg/ml)	13.0 [10-26.3] (n =20)	11.3 [10.0-20.5] (n =23)	0.701
Cortisol (ug/dl)	17.5 ± 4.9 (n = 20)	17.5 ± 7.4 (n =23)	0.986
UFC (ug/24h)	328 [262-698] (n = 19)	379.0 [198.1-879.0] (n=23)	0.850
DHEA-S (ng/ml)	193.0 [82.5-244.5] (n=11)	465.5 [266.0-1824.0] (n=22)	0.061
Urine VMA (mg/24h)	2.8 ± 1.3 (n=18)	3.0 ± 1.1 (n=17)	0.566
Tumor size (cm)	3.0 [2.5-4.0]	3.5 [3.2-4.1]	0.061

Discussion

Somatic mutations in the hotspot mutation Leu206Arg of *PRKACA* gene occurred in 45.5% of patients with unilateral adenomas with overt Cushing's syndrome, which is comparable to the numbers previously reported by two other Asian studies; 65% by Cao et al. (3) and 52% by Sato el al. (12) Considering that our cohort does not include patients with subclinical Cushing's syndrome, the frequency could be higher, even though subclinical Cushing's syndrome is apparently less associated with the mutation (1,12). It seems likely that Asian patients have a higher frequency of *PRKACA* gene mutation, compared to western patients (1,2,13).

Unlike previous reports (1,12,13), *PRKACA* L206R mutation in our cohort was not associated with biochemical markers of cortisol production. However, regarding tumor size, patients with the mutations tended to have smaller adenomas, and also more patients had comorbidities such as hypertension and diabetes. *PRKACA* mutation is considered sufficient on its own to cause autonomous cortisol production in adrenal adenomas (2). Primarily in agreement with this statement, our study suggests that there may be other causes along the cAMP-PKA pathway that causes hypercortisolism in adrenal Cushing's disease patients, in combination with the *PRKACA* mutation. Further analyses on mutations of genes such as GNAS, PDE11A, PRKAR1A are warranted in Korean patients on this matter, in addition to germline mutational analyses.

One unique finding of this study is that patients with *PRKACA* mutation

had lower levels of DHEA-S. DHEA-S is made exclusively by the adrenal glands, primarily in the zona reticularis layer of the adrenal cortex (18). It is used as a biomarker to rule out ovarian or testicular origin or excess androgen. Normal blood levels of DHEA-S can differ by sex and age. Most of the patients without *PRKACA* mutation in our cohort were within normal ranges (350-4300 ng/ml) of DHEA-S. Also, DHEA-S levels in most patients with *PRKACA* mutation did not reach the lower limit. Lower levels of DHEA-S are often considered a marker of adrenal Cushing's syndrome, especially since it is directly regulated by ACTH levels (19). However, the wide normal range of DHEA-S makes it a poor diagnostic tool in determining the cause of hypercotisolism (20).

Another interesting finding from this study is the association between *PRKACA* L206R mutation and clinical features. Patients with *PRKACA* mutation had less association with amenorrhea, compared to those without mutation. Amenorrhea results from the decrease in gonadotropin-releasing hormone (GnRH) release from the hypothalamus, caused by the negative feedback of high cortisol level (14). *PRKACA* mutation could have a part somewhere in this pathway to have a protective effect in female patients with adrenal Cushing's syndrome. There was a tendency for patients with *PRKACA* mutation to present with central obesity, buffalo humps, and specific skin problems. *PRKACA* mutation validation in a larger cohort of patients could further define these characteristics.

Furthermore, patients with the wild type *PRKACA* gene had a tendency to show signs of androgen excess, such as oily skin, hirsutism and amenorrhea; all of which are concordant with the effects of DHEA-S. However, as

aforementioned, normal ranges of DHEA-S level vary according to sex and age, and are also ACTH dependent. Not all patients with ACA showed low levels of serum DHEA-S in our study. The difference in DHEA-S levels and in clinical features between adrenal Cushing's syndrome patients with *PRKACA* mutation and those without the mutation suggests that the mechanisms of *PRKACA* mutation could be complexly involved within the entire adrenal steroidogenesis pathway.

The main limitation of this study is the small number of samples that was included for final analysis. Some of the FFPE blocks yielded with inadequate DNA quantity and quality. Most of the samples that were unsuccessful for *PRKACA* sequencing had less than 20ng/uL DNA. Analysis with fresh tissue samples could refine and improve our results.

In conclusion, *PRKACA* mutation could be complexly associated with the entire adrenal steroidogenesis pathway. Adrenal Cushing's syndrome patients with this mutation have lower levels of serum DHEA-S; show specific clinical features such as central obesity, buffalo hump, and dry skin; and also seem to have some kind of protection against amenorrhea. We suggest the presence of another pathway in which *PRKACA* impacts on these outcomes. In this sample of patients, *PRKACA* mutation was not associated with any difference in biochemical features compared to those without mutation. Further analysis of a larger number of patients is warranted.

References

1. Beuschlein F, Fassnacht M, Assie G, Calebiro D, Stratakis CA, Osswald A, et al. Constitutive Activation of PKA Catalytic Subunit in Adrenal Cushing's Syndrome. N Engl J Med. 2014;370:1019-28.

2. Goh G, Scholl UI, Healy JM, Choi M, Prasad ML, Nelson-Williams C, et al. Recurrent activating mutation in *PRKACA* in cortisol-producing adrenal tumors. Nat Genet. 2014;46(6):613-7.

3. Cao Y, He M, Gao Z, Peng Y, Li Y, Li L, et al. Activating hotspot L205R mutation in *PRKACA* and adrenal Cushing's syndrome. Science. 2014;344(6186):913-7.

4. Nieman LK, Biller BM, Findling JW, et al. The diagnosis of Cushing syndrome: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2008;93:1526-40.

5. Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's Syndrome. Lancet 2006;367:1605-17.

6. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. Science. 2011;331(6018):768-72.

7. Lacroix A, Bourdeau I, Lampron A, Mazzuco TL, Tremblay J, Hamet P. Aberrant G-protein coupled receptor expression in relation to adrenocortical overfunction. Clin Endocrinol (Oxf) 2010;73:1-15.

8. Fragoso MC, Domenice S, Latronico AC, et al. Cushing's syndrome secondary to adrenocorticotropin-independent macronodular adrenocortical

hyperplasia due to activating mutations of GNAS1 gene. J Clin Endocrinol Metab 2003;88:2147-51.

9. Cazabat L, Ragazzon B, Groussin L, Bertherat J. PRKAR1A mutations in primary pigmented nodular adrenocortical disease. Pituitary 2006;9:211–9.

10. Horvath A, Boikos S, Giatzakis C, Robinson-White A, Groussin L, Griffin KJ, et al. A genome-wide scan identifies mutations in the gene encoding phosphodiesterase 11A4 (PDE11A) in individuals with adrenocortical hyperplasia. Nature genetics 2006;38(7):794-800.

11. Lodish MB, Yuan B, Levy I, Braunstein GD, Lyssikatos C, Salpea P, et al. Germline *PRKACA* amplification causes variable phenotypes that may depend on the extent of the genomic defect: molecular mechanisms and clinical presentations. European Journal of Endocrinology. 2015;172:803-11.

12. Sato Y, Maekawa S, Ishii R, Sanada M, Morikawa T, Shiraishi Y, et al. Recurrent somatic mutations underlie corticotropin-independent Cushing's syndrome. Science. 2011;344(6186):917-20.

13. Thiel A, Reis AC, Haase M, Goh G, Schott M, Willenberg HS et al. *PRKACA* mutations in cortisol-producing adenomas and adrenal hyperplasia: a single-center study of 60 cases. European Journal of Endocrinology. 2015;172:677-685.

14. Lado-Abeal J, Rodriguez-Arnao J, Newell-Price JD, Perry LA, Grossman AB, Besser GM, et al. Menstrual abnormalities in women with Cushing's disease are correlated with hypercortisolemia rather than raised circulating androgen levels. J Clin Endocrinol Metab 1998;83:3083-8.

15. Zeiger MA, Thompson GB, Duh QY, Hamrahian AH, Angelos P, Elaraj D, et al. American Association of Clinical Endocrinologists and American Association of Endocrine Surgeons Medical Guidelines for the Management of Adrenal Incidentalomas. Endocr Pract 2009;15(Suppl 1)

16. Etxabe J, Vazquez JA. Morbidity and mortality in Cushing's disease: an epidemiological approach. Clin Endocrinol (Oxf) 1994; 40: 479-84.

17. Stratakis CA, Boikos SA. Genetics of adrenal tumors associated with Cushing's syndrome: a new classification for bilateral adrenocortical hyperplasias. Nature 2007;3(1):748-57.

18. Auchus RJ. The Physiology and Biochemistry of Adrenarche. Endocr Dev. 2011;20:20-7.

19. Flecchia D, Mazza E, Carlini M, Biatto A, Olivieri F, Serra G, et al. Reduced serum levels of dehydroepiandrosterone sulphate in adrenal incidentalomas: a marker of adrenocortical tumour. Clin Endocrinol (Oxf). 1995;42(2):129-34.

20. Hong AR, Kim JH, Hong ES, Kim IK, Park KS, Ahn CH, et al. Limited Diagnostic Utility of Plasma Adrenocorticotropic Hormone for Differentiation between Adrenal Cushing Syndrome and Cushing Disease. Endocrinol Metab 2015;30:297-304.

요약 (국문초록)

서론: cyclic AMP (cAMP)-단백질인산화효소 A (protein kinase A, PKA) pathway에서 cAMP signaling의 증가로 인해 코티졸 생성의 증가 되어, 부신성 쿠싱 증후군이 발생하게 된다. PKA의 알파 촉매 소단위체 (catalytic subunit alpha)를 코딩하는 *PRKACA* 유전자의 체세포 및 생식세포 돌연변이가 최근 쿠싱 증후군을 유발하는 35~65.5%의 부신 선종에서 발견되었다. 이 연구의 목적은 한국인 환자에서 Leu206Arg (c.617A→C) hotspot mutation을 확인하고, 그에 따른 임상적인 특성을 분석하는 것이다.

방법: 2000년 1월부터 2013년 12월까지 서울대학교병원에서 명확한 증상의 (overt) 부신성 쿠싱 증후군으로 부신 절제술을 시행 받은 환자 68명을 대상으로 후향적인 연구를 시행하였다. 55명의 환자의 formalin fixed paraffin-embedded (FFPE) 블록에서 DNA를 검출하여 *PRKACA* sequencing을 시행하였다.

결과: Hotspot mutation Leu206Arg에 대한 *PRKACA* sequencing은 55개의 DNA 샘플 중 48 (87.3%)개 에서 성공적으로 이루어졌다. Leu206Arg *PRKACA* 돌연변이는 부신 선종 환자 중 20명 (45.5%)에서 발견되었다. *PRKACA* 돌연변이가 있는 환자들은 없는 환자들에 비해 DHEA-S 수치가 의미 있게 낮았다 (중앙값 193.0 ng/ml vs. 473.0 ng/ml, p=0.001). 돌연변이가 있는 선종의 환자들은 나이가 더 많은 경향을 보였고 (43.9 ± 12.2 vs. 39.5 ± 12.1 years), 고혈압과 당뇨와 같은 공병을 앓고 있을 경향이 있었다. *PRKACA* 돌연변이가 있는 부신 선종은 돌연변이가 없는 선종보다 크기가 작았다 (3.2 ± 0.9 vs. 3.8 ± 1.1cm).

결론: *PRKACA* 돌연변이는 부신에서의 스테로이드 합성과정에 복

잡하게 관여하고 있는 것으로 보인다. 부신성 쿠싱 증후군 환자에서 이 돌연변이는 혈청 DHEA-S 수치의 저하와, 복부비만, buffalo hump, 피부 건조 등과 같은 특이적인 증상과 연관이 있을 수 있겠다. 무월경에 대해서는 보호 역할을 하고 있는 듯 하다. 이 연구의 환자 군에서는 *PRKACA* 돌연변이는 생화학적인 지표들과는 아무 관련이 없었다. 더 많은 환자를 대상으로 *PRKACA* 돌연변이 분석을 하는 것이 도움이 되겠다.

주요어: 쿠싱 증후군, 부신 선종, *PRKACA* 돌연변이

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