KCNJ5 mutations in aldosterone- and cortisol-co-secreting adrenal adenomas

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Abstract. Adrenal aldosterone-producing adenomas (APA) are rarely associated with the clear co-secretion of cortisol. Somatic mutations of the potassium channel KCNJ5 gene, with the hotspots G151R and L168R, have been recently identified in patients with APA. However, whether APAs that secrete cortisol have these mutations remains unclear. We examined three patients with APAs showing clear autonomous secretion of cortisol who possessed a 1 mg dexamethasone suppression test (DST) with a failure of the serum cortisol level to drop below 3.0 µg/dL, a morning plasma ACTH level of less than 10 pg/mL, and suppressed accumulation in the intact adrenal on 131I- adosterol scintigraphy, or postoperative adrenal insufficiency. Laparoscopic adrenectomy revealed all tumors to be golden yellow, and histological examination confirmed them to be adrenocortical adenomas. All these patients required replacement therapy with hydrocortisone after surgery. Sequencing demonstrated that 2 of 3 cases showed a mutation of the KCNJ5 gene, one with c.451G>A, p.G151R and one with c.503T>G, p.L168R. Furthermore, the mRNA levels of steroidogenic enzymes including CYP11B1, CYP11B2, HSD3B2, CYP17A1, CYP11A1 and KCNJ5 in the 3 cases did not differ from those in 8 pure APAs not showing any of the above conditions for autonomous cortisol secretion. In addition, all 8 pure APAs harbored mutations of the KCNJ5 gene. These findings suggested that at least some aldosterone- and cortisol-co-secreting adrenal tumors have mutations of the KCNJ5 gene, supporting the origin to be APA, and pure APAs may show a high incidence of KCNJ5 mutations.

Key words: KCNJ5, Aldosterone-producing adenoma, Adrenocortical adenomas, Co-secretion of cortisol
We and others have recently confirmed the same two mutations (G151R and L168R) of the KCNJ5 gene in APAs in Japan, Australia, the United Kingdom, and Europe. We found mutations of the KCNJ5 gene in 15 of 23 (about 65%) APAs in Japan, and Azizan et al. reported a lower frequency, 38% in Australia and 44% in the United Kingdom [11, 12]. Boulkroun et al. examined the largest number, 380 cases of APA, and found that 34% patients had mutations in Europe [13].

In the present study, since the concept of preclinical (or subclinical) Cushing’s syndrome is not universally accepted [14], we selected cases who possessed all following conditions; 1 mg dexamethasone suppression test (DST) with a failure of the serum cortisol level to drop below 3.0 µg/dL, a morning plasma ACTH level of less than 10 pg/mL, and suppressed accumulation in the intact adrenal on [131] I-adosterone scintigraphy, or postoperative adrenal insufficiency. We then examined whether these APAs associated with the clear co-secretion of cortisol have mutations of the KCNJ5 gene, and measured mRNA levels for several steroidogenic enzymes and compared them to those in pure APAs not showing any above parameters for autonomous cortisol secretion.

Subjects and Methods

Subjects

Each subject provided written informed consent, and the study was approved by the ethics committee on human research of Gunma University. The diagnosis of PA was performed as reported previously [1, 9, 15].

RNA extraction and detection of mutations of KCNJ5 cDNA by direct sequencing

All specimens of APA and some normal parts of the adrenal cortex were frozen in liquid nitrogen immediately after removal during the operation. Total RNA was prepared, and cDNA was reverse-transcribed and sequenced with specific primers sets for the entire KCNJ5 cDNA as reported previously [11].

Expression of KCNJ5 and steroidogenic enzyme mRNA levels determined by Real-time PCR

To measure the level of KCNJ5 and steroidogenic enzyme mRNA in each adenoma, 0.5 µL of cDNA was subjected to real-time PCR as reported [16, 17]. All reactions were performed in triplicate using TaqMan probes and an Applied Biosystems 7500 sequence detection system. The TaqMan probes for KCNJ5 (Hs00168476_m1), GAPDH (Hs99999905_g1), cholesterol side-chain cleavage enzyme, CYP11A1 (Hs00167984_m1), CYP11B1 (Hs01596404_m1), CYP11B2 (Hs01597732_m1), 3β-hydroxysteroid dehydrogenase (HSD3B2) (Hs00605123_m1), and 17α-hydroxylase (CYP17A1) (Hs01124136_m1) were from Applied Biosystems. The level of each mRNA relative to that of GAPDH was calculated using a standard curve, and relative quantification was performed as described in ABI User Bulletin #2. All experiments were repeated at least twice. The level of mRNA expression for each patient was evaluated with the median value for the normal adrenal cortex set as 1.0.

Hormone measurement

Plasma aldosterone levels were measured in most cases 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.

Statistical analysis

All results are expressed as the median or mean ± SD for continuous variables. Group comparisons were performed with an ANOVA and Student’s t test for normally distributed data, or the Wilcoxon rank-sum test or Mann-Whitney test for non-normally distributed data for continuous variables. All tests for significance and resulting P values were two-sided, with a level of significance of 5%. Statistical analyses were performed using JMP 5.1.2 (SAS Institute Inc. Cary, NC, USA).

Results

Case reports

Case 1. A 51-year-old woman had hypertension and hypokalemia with 2.2 mEq/L and a symptom of Cushing’s syndrome, moon face. Her plasma aldosterone concentration (PAC) was 133.0 ng/dL, plasma renin activity (PRA), 0.6 ng/mL/hr; morning serum cortisol level, 31.2 µg/dL; serum ACTH level, < 5 pg/mL. She showed suppressed accumulation in the intact adrenal on scintigraphy. Her midnight cortisol level at 23:00 pm was 12.3 µg/dL. After a 1 mg DST, serum cortisol level was 32 µg/dL. A CT scan demonstrated a tumor 4.5 cm in diameter in the left adrenal gland. The maximum standardized uptake value (maxSUV) on F-18 fluorodeoxyglucose-positron emission tomography (FDG-PET) was 4.08.
Case 2. A 48-year-old woman had hypertension and hypokalemia with 3.4 mEq/L, but did not have any symptoms of Cushing’s syndrome. Her PAC was 23.1 ng/dL, PRA, 0.4 ng/mL/hr, serum cortisol level, 9.9 µg/dL, and serum ACTH level, 6.8 pg/mL. She showed suppressed accumulation in the intact adrenal on scintigraphy. Her midnight cortisol level was 5.0 µg/dL. After a 1 mg DST, her serum cortisol level after a 1mg dexamethasone suppression test. SBP and DBP, systolic and diastolic blood pressure. The doses of Spironolactone and potassium (K) supplement taken before surgery are indicated. All the patients were given spironolactone for at least one week before the surgery. Tumor size is the maximum diameter of the tumor observed in the CT study. Scintigraphy, suppressed accumulation in the intact adrenal gland. G151RG/A represents a heterozygous mutation of c.451G>A (p.G151R) and L168R for c.503T>G (p.L168R). WT represents wild-type alleles. Pt. 3 showed insufficiency of adrenocortical function after the surgery.

Furthermore, histological examination confirmed that all three APAs (Cases 1–3) were isolated adenomas and there was no additional micro-cortisol producing adenoma.

Characteristics of patients with APA (pure APA) not showing any criteria indicating autonomous cortisol secretion

We had 8 operated samples of APAs showing no parameters of autonomous cortisol secretion including the 1 mg DST with a failure to suppress the serum cortisol level to less than 3.0 µg/dL, a morning plasma ACTH level to less than 10 pg/mL, and the accumulation of intact adrenal on scintigraphy or post-operated adrenocortical insufficiency. We also excluded cases who had missed any value for these parameters. We defined these APAs as pure APAs in the present study. Six of 8 patients correspond to Pt. 2, 3, 9, 10, 12 and 13 in Table 1 in our previous study [11]. The additional 2 cases were a 50-year-old male, and a 36-year-old female, and their PAC, PRA and serum potassium levels were 94.4 and 54.2 ng/dL, 0.1 and 0.2 ng/mL/hr, and 2.6 and 2.5 mEq/L, respectively. The mean age of these 8 patients was 42 ± 13 years, and 38 percent were male. The mean PAC was 35.4 ± 17.1 ng/dL (17.6~94.4), PRA, mean 0.4 ± 0.3 ng/mL/hr (0.1~0.9) and the mean serum potassium level, 2.7 ± 0.6 mEq/L (2.1~3.5). The maximum tumor diameter measured by CT was 1.5 ± 0.4 cm (1.0~2.3).

Mutations of KCNJ5 in APAs showing autonomous cortisol secretion and pure APAs

Analysis of the KCNJ5 cDNA in APAs demonstrated that patient 1 had somatic mutations of the KCNJ5
cortisol, to be increased in APAs showing co-secretion of cortisol. Therefore, we examined the expression of \textit{CYP11B1} and other steroidogenic enzymes mRNA including \textit{CYP11B2}, \textit{CYP17A1}, \textit{HSD3B2}, \textit{CYP11A1} as well as \textit{KCNJ5}. In Fig. 1, black circles indicate values of the patients with APAs showing co-secretion of cortisol and gray dots, pure APAs. 

\textbf{Expression of steroidogenic enzymes and \textit{KCNJ5} mRNA in patients with APAs showing autonomous cortisol secretion and pure APAs}

We expected a steroidogenic enzyme such as \textit{CYP11B1}, which is responsible for the production of cortisol, to be increased in APAs showing co-secretion of cortisol. Therefore, we examined the expression of \textit{CYP11B1} and other steroidogenic enzymes mRNA including \textit{CYP11B2}, \textit{CYP17A1}, \textit{HSD3B2}, \textit{CYP11A1} as well as \textit{KCNJ5}. In Fig. 1, black circles indicate values of the patients with APAs showing co-secretion of cortisol and indicate the corresponding patient number in Table 1, while gray circles represents those of pure APAs. For all steroidogenic enzyme and \textit{KCNJ5} mRNA levels, the value for the normal adrenal gland adjust to the APA was set as 1.0. As shown in Fig. 1A, as expected, levels of \textit{CYP11B2} mRNA in most tumors including 3 APAs with co-secretion of cortisol were higher than or at least similar to those in normal adre-
In the present study, 2 of 3 APAs showing clear co-secretion of cortisol had a mutation of the \textit{KCNJ5} gene. Boulkroun \textit{et al.} recently examined 16 patients with cortisol-producing adenomas (CPA) who showed typical symptoms of Cushing’s syndrome and found no mutations of the \textit{KCNJ5} gene [13]. We also examined 6 cases of CPA (submitted), but they did not have the mutation. Therefore, although the number of CPAs examined was small, considering the high prevalence (at least 30–%) of the mutation of the \textit{KCNJ5} gene in APA in several countries, the mutation may be highly specific to aldosterone-producing adenomas and familial aldosteronism type III. Therefore, at least some of the APAs with co-secretion of cortisol appeared to have mutations of KCNJ5, suggesting them to be derived from APA, not from CPA. To confirm this, we performed qPCR for several steroidogenic enzymes, which may reflect their origin of the zone in the adrenal gland. However, the mRNA levels of steroidogenic enzymes of 3 APAs with co-secretion of cortisol did not show any specific profile. Hiraishi \textit{et al.} also reported that in a histopathological and immunohistochemical study of 3β-HSD and CYP17, CYP11B2 mRNA levels in APAs with subclinical Cushing’s syndrome diagnosed with the guideline of a Research Committee for Adrenal Diseases supported by the Japanese Ministry of Health, Labor and Welfare did not show any difference from those in APAs [18].

Conversely, it is of interest that all 8 pure APAs harbored a \textit{KCNJ5} gene mutation, who did not show any parameter for autonomous cortisol secretion such as the 1 mg DST with a failure of the serum cortisol level to drop to less than 3.0 μg/dl, a morning plasma ACTH level of less than 10 pg/ml, and suppressed accumulation of intact adrenal in scintigraphy or postoperative adrenal insufficiency. In a previous study, we found about 65 % of 23 Japanese patients with APA to have a somatic mutation of the \textit{KCNJ5} gene [11]. Among these 23 patients, 6 had pure APAs as defined in the present study, and all of which had the mutation of the \textit{KCNJ5} gene. Monticone \textit{et al.} recently reported a study of steroidogenic enzyme mRNA levels in 47 APAs including 5 Japanese patients, and found all 5 cases to have a mutation of the \textit{KCNJ5} gene [19]. Although the details for these 5 cases were not reported, Japanese patients with typical APA may have a high prevalence of somatic mutations of the \textit{KCNJ5} gene. A larger scale study is required to reach a conclusion as to the precise prevalence of mutations of the \textit{KCNJ5} gene in Japanese with pure APAs.

Among patients with adrenocortical adenomas, those with no typical symptoms of Cushing’s syndrome (CS) such as moon face, buffalo hump etc. but showing the autonomous secretion of cortisol have been identified as having preclinical (or subclinical) Cushing’s syndrome [20, 21]. Although the concept of preclinical (or subclinical) CS is not universally accepted [14], Spath \textit{et al.} recently reported that we should consider an APA with co-secretion of cortisol if a patient has 1) PA and an adenoma that is larger than 2.5 cm, 2) cortisol that is non-suppressible with overnight low-dose dexamethasone, or 3) grossly elevated serum levels of hybrid steroids, such as 18-OH-F [22]. In fact, although we did not have a chance to measure serum hybrid steroids, all three cases had a tumor larger than 2.5 cm. These findings further support that the larger the APA, the higher the frequency of co-secretion of cortisol.

The exact prevalence and clinical and pathological features of APA with co-secretion of cortisol have not been fully elucidated. Hogan \textit{et al.} first reported an aldosterone- and cortisol-producing adenoma in 1977 [23]. Subsequently, several papers have been published regarding the association of PA with the autonomous secretion of cortisol [22, 24, 25]. Many such reports have come from Japan or Europe. Piaditis \textit{et al.} have reported that PA with hypercortisolism was observed in 12.1% of 83 adrenal incidentalomas [26]. In Japan, the prevalence was reported to be 15–30% [9, 18, 27]. Although the reason for the high prevalence of an association with autonomous cortisol secretion in APAs remains unclear, many cases have been described by Japanese investigators and this may have led to greater awareness in Japan [22]. Further study is required to investigate the regional difference in the prevalence of APA with co-secretion of cortisol and to establish a universal concept of subclinical Cushing’s syndrome.
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Disclosure Summary

All authors have nothing to disclose.

References


