Prevalence of Somatic *KCNJ5* Mutations in Thai Patients With Aldosterone-Producing Adrenal Adenomas

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Somatic *KCNJ5* mutations result in excess aldosterone production and are reported to be more common in Asia than elsewhere. To assess the prevalence of somatic *KCNJ5* mutations in Thai patients with aldosterone-producing adrenal adenomas (APAs) in a single tertiary center, we analyzed the paraffin-embedded tissue of *KCNJ5* mutations from 96 patients with sporadic APAs who underwent unilateral laparoscopic adrenalectomy at our center during 2007 to 2016. We also assessed the clinical characteristics, treatment outcomes, and biochemistry and histologic differences among patients with and without somatic *KCNJ5* mutations. Of the 96 patients with APA, 67 (70%) had somatic mutations of the *KCNJ5* gene: 39 patients with p.G151R, 26 patients with p.L168R, one patient with p.T158A, and one patient with p.W126R. All patients presented with hypertension. Hypokalemia was documented in 98% of patients. The hypertension cure rate at 1 year after surgery was 35%. Patients with somatic *KCNJ5* mutations required more potassium supplementation and had adrenal histology compatible with zona fasciculata–like cells compared with patients without the mutations (all $P < 0.05$). There were no significant differences in preoperative plasma aldosterone concentration (PAC), plasma renin activity, aldosterone/renin ratio, potassium level, treatment of hypertension, tumor size, and hypertension cure rate among patients in the *KCNJ5*-mutant and nonmutant groups. In a multivariate analysis, a higher PAC was associated with the presence of somatic *KCNJ5* mutations. In summary, the prevalence of somatic *KCNJ5* mutations in patients with sporadic APAs in Thailand, an Asian country with residents of different ethnic backgrounds, is comparable to previous reports in Asia.

Freeform/Key Words: aldosterone-producing adrenal adenomas, *KCNJ5* somatic mutations, Thai patients

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Aldosterone-producing adrenal adenomas (APAs) account for ~30% of the cases of primary aldosteronism [1]. The majority of cases of APA are sporadic, with <5% being familial cases [2]. The underlying mechanism of excess aldosterone production results from abnormalities

in either K^+, Na^+ K^+-ATPase, or Ca^{2+} channels in zona glomerulosa (ZG) cells of adrenal glands [3]. Choi *et al.* [4] discovered the mutations in the *KCNJ5* gene, which encodes the Kir3.4 K^+ channel that causes the loss of K^+ channel selectivity. Consequently, Na^+ influx through mutated K^+ channels leads to ZG cell membrane depolarization and increased intracellular Ca^{2+} by the opening of voltage-gated Ca^{2+} channels, which activates aldosterone synthesis by increasing transcription and translation of CYP11B2 (aldosterone synthase) [5]. Mutation of this gene is most commonly found among patients with sporadic APAs [6]. Many different *KCNJ5* mutations causing sporadic APAs have been reported. The most common mutations are the substitutions p.Gly151Arg or p.Leu168Arg. Other mutations are p. Thr158Ala, p.Trp126Arg, and p.Ile157Del.

The exclusive site of aldosterone production in an adult adrenal gland is the ZG. However, ZG-like APAs are less abundant than zona fasciculata (ZF)-like APAs [7, 8]. Monticone *et al.* [9] demonstrated that 90% of tumors with *KCNJ5* mutations were composed of mainly ZF-like cells. Most tumors with either no mutation or with mutations in *ATP1A1-ATP2B3-CACNA1D* were composed of ZG-like cells.

Apart from histological differences, patients harboring *KCNJ5*-mutated APAs have clinical and biochemical characteristics that are different from patients without somatic *KCNJ5* mutations. A meta-analysis by Lenzini *et al.* [6] demonstrated that patients with *KCNJ5*-mutated APAs were younger, were predominantly female, and had higher plasma aldosterone concentrations (PACs) and larger tumors compared with patients without somatic *KCNJ5* mutations. This meta-analysis also showed that *KCNJ5* mutations were more common in patients from Asia than from elsewhere (63% vs 35%; $P < 0.003$). Recent studies in Taiwanese patients supported this information, with a prevalence of somatic *KCNJ5* mutations of ~62% [10]. The study by Lenzini *et al.* [6] also reported an association of urinary sodium excretion and prevalence of somatic *KCNJ5* mutations. However, the reason why this mutation is more common in Asia remains unclear.

In Thailand, the prevalence of somatic *KCNJ5* mutations in patients with sporadic APAs has not been determined. The aims of this study were (1) to determine the prevalence of somatic *KCNJ5* mutations in patients with sporadic APAs; (2) to discover the clinical, biochemical, and histopathological correlation and surgical outcomes in terms of resolution of hypertension in patients with different mutation status; and (3) to determine clinical risk factors associated with somatic *KCNJ5* mutations.

1. Subjects and Methods

A. Patients

We performed a retrospective evaluation of patients with APAs who underwent unilateral laparoscopic adrenalectomy at King Chulalongkorn Memorial Hospital (KCMH), which is a large tertiary center in Bangkok, during 2007 to 2016. All patients visited the outpatient clinic at KCMH and completed the evaluation leading to the diagnosis of APA. The diagnostic process of APA was formulated according to the recommendations of the current guidelines [11]. All patients underwent unilateral adrenalectomy in the Department of Urology at KCMH. Adrenal tissues were preserved and collected as paraffin-embedded tissue for the mutation analysis. All subjects who enrolled after the approval of the local ethics committee provided informed consent.

Patients over the age of 18 years who underwent unilateral adrenalectomy at KCMH during 2007 to 2016 and had adrenal tissue for mutation analysis were included in the study. Patients with persistent hyperaldosteronism after unilateral adrenalectomy were excluded. Persistent hyperaldosteronism was defined by persistent hypertension with high PAC, aldosterone/renin ratio (ARR), and suppressible plasma renin activity (PRA). Persistent hypertension was defined by a recorded office systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg. High PAC was defined as value >15 ng/dL. High ARR was defined as value of >20. PRA of <1 ng/ mL/h was considered as suppressed PRA. Adrenal tissue with possible DNA damage that could not be amplified for successful PCR was excluded.

B. Definitions and Outcome Variables

We determined postoperative hyperkalemia as a serum potassium of >5.0 mmol/L without potassium supplementation or medications that could explain the presence of hyperkalemia. Postoperative transient hyperkalemia was defined as hyperkalemia that occurred within the first 3 months after adrenalectomy and resolved spontaneously after 3 months. Postoperative persistent hyperkalemia was defined as hyperkalemia that lasted >3 months after adrenalectomy. An estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease Study Equation [13]. Blood pressure was documented during an outpatient visit. Resolution of hypertension was defined as a recorded office systolic blood pressure of <140 mm Hg and diastolic blood pressure of <90 mm Hg without the use of antihypertensive agents.

C. DNA Extraction and KCNJ5 Sequencing

All adrenal glands included in the study were formalin-fixed paraffin-embedded (FFPE), cut into 4-μm slices, and stained with hematoxylin and eosin. Histological examination to determine the site of tumors was performed by an experienced pathologist. Deparaffinization was done by using xylene and absolute ethanol. Tumor DNA was then extracted using QIAamp DNA FFPE tissue (Qiagen, Gmbh, Hilden, Germany). DNA concentration and purity were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Willington, DE). Absorbance at 260 nm was measured. The entire coding sequence (exons 2 and 3) was amplified and sequenced using gene-specific primers as previously reported [14]. Direct sequencing of PCR products was then performed (1st BASE DNA Sequencing, Selangor, Malaysia).

D. Pathological Analysis

Cellular composition was determined by examining for features of ZF (large, lipid-laden clear cells with round to oval vesicular nuclei), ZG (small, compact cells with high nuclear/cytoplasmic ratio and a moderate amount of lipid), and zona reticularis (lipid-sparse cytoplasm, compact) cells. The tumor was classified as ZF-like when the percentage of large vacuolated cells was >50%. If the percentage of ZF-like cells was <50% and ZG-type cells were the most prominent cell type, the tumor was classified as ZG-type [9]. The analysis was done by a single experienced pathologist.

E. Statistical Analysis

Continuous variables were presented as median or mean $\pm$ SD. Within-group changes from baseline to follow-up were assessed by paired t test. Group comparisons were examined with an independent t test or χ^2 tests for normally distributed data or with Mann-Whitney U test for nonparametric testing. Multivariate logistic regression was used to identify independently significant risk factors associated with the presence of KCNJ5 mutations. In a univariate analysis, a P value of <0.20 was considered statistically significant. Factors significantly associated with the presence of mutations in the univariate logistic regression were inserted into a multivariate model. Time to resolution of hypertension after adrenalectomy was analyzed using the Kaplan-Meier survival curve, and data between groups were compared using the log-rank test. A P value <0.05 was considered statistically significant. Statistical analysis was performed with SPSS Statistics version 22.0.

2. Results

From January 2007 to December 2016, 96 patients [59 women (61%)] met our inclusion criteria (Fig. 1). At 1 year after adrenalectomy, 58 patients were either lost to follow-up or discharged from the clinic (60%). The median follow-up time was 8.5 months [interquartile
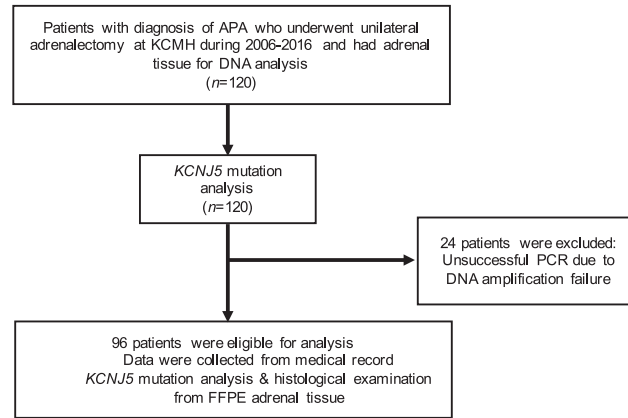
Figure 1. Flowchart of the study patient selection.

range (IQR), 2 to 21 months]. All patients presented with hypertension. Other comorbidities were diabetes mellitus, prediabetes, dyslipidemia, obstructive sleep apnea, and history of cerebrovascular disease (Table 1). Two patients had suffered stroke. The first case was a 68-year-old woman with hypertension and chronic atrial fibrillation. At the age of 63, she was diagnosed with embolic stroke. The other case was a 75-year-old man with hypertension and dyslipidemia.

Table 1. Clinical and Biochemical Characteristics of Patients in the Study

Variables	Value
Age, y[a]	47.80 ± 12.04
Female, n (%)	59 (62)
Weight, kg[a]	66.55 ± 15.35
BMI, kg/m^{2a}	25.64 ± 5.50
Comorbidity, n (%)	
Hypertension	96 (100)
Dyslipidemia	24 (25)
Dysglycemia	33 (34)
Diabetes mellitus	10 (10)
Impaired fasting glucose or prediabetes	23 (24)
Cerebrovascular disease	2 (2)
Hypokalemia, n (%)	94 (98)
Daily potassium supplement, mEq/d[b]	85 (40–120)
Antihypertensive agents, n (%)	
Total CCBs	85 (89)
Nondihydropyridines CCBs	59 (61)
Dihydropyridines CCBs	31 (32)
α-Blockers	67 (70)
Vasodilators	35 (36)
Mineralocorticoid antagonists	8 (8)
Preoperative antihypertensive agents, n[a]	2.22 ± 0.86
PAC, ng/dL[b]	50.60 (30.30–73.00)
PRA, ng/mL/h[b]	0.37 (0.20–0.66)
ARR[b]	116.67 (64.87–274.50)
eGFR, mL/min/1.73 m^{2a}	87.20 ± 20.97
Preoperative potassium level, mmol/L[a] (3.6–5.0)	3.64 ± 0.59
Lowest potassium level, mmo/L[a] (3.6–5.0)	2.62 ± 0.58
Tumor size, cm[b]	1.5 (1.2–2.0)
Duration of hypertension, mo[b]	51 (24–120)
Duration of follow-up, mo[b]	8.50 (2.00–21.00)

Abbreviations: BMI, body mass index; CCB, calcium channel blocker; MAR, mineralocorticoid antagonist.
[a]Data presented as mean ± SD.
[b]Data presented as median (IQR).

He was diagnosed with right middle cerebral artery infarction when he was 70 years old. Genetic testing for the chimeric CYP11B1/B2 was not done in these two individuals.

The median time from the onset of hypertension to the diagnosis of primary aldosteronism was 51 months (IQR, 24 to 120). Hypokalemia was documented in 98% of the patients. Median levels of PAC, PRA, and ARR were 50.60 ng/dL, 0.37 ng/mL/h, and 116.67, respectively. Confirmatory tests were done in 79 patients (82%). Saline infusion test in sitting position was the most commonly used procedure. Findings from adrenal CT or MRI were used in conjunction with or without adrenal venous sampling (AVS) to indicate unilateral aldosterone excess in all patients. Among the 11 patients (11%) who underwent AVS, the imaging showed either bilateral enlarged adrenal glands or small adrenal nodule, whereas 98% of patients who did not undergo AVS had unilateral enlarged adrenal glands with a normal-appearing contralateral gland.

The most common antihypertensives used were calcium-channel blockers, α-blockers, and vasodilators in 89%, 70%, and 36% of patients, respectively, and 8% of patients used preoperative mineralocorticoid antagonists. Thirty patients (31%) used more than two antihypertensive medications. The mean number of antihypertensive agents was 2.22 ± 0.86.

A. Somatic KCNJ5 Mutations in Patients With APAs

Sequence analysis of *KCNJ5* demonstrated the presence of four different somatic mutations in 67 patients (70%). Thirty-nine patients (41%) had p.Gly151Arg mutations, and 26 patients (27%) had p.Leu168Arg mutations. One patient had the p.Thr158Ala mutation, and another patient had the p.Trp126Arg mutation.

B. Clinical and Biochemical Phenotypes in Relation to KCNJ5 Mutation Status

When comparing clinical and biochemical phenotypes of patients characterized by mutation status, the *KCNJ5*-mutated group required substantially more potassium supplementation compared with the non–*KCNJ5*-mutated group (115 vs 80 mEq/d; P = 0.022). There was no significant difference in terms of sex, age, duration of hypertension, PAC, PRA, ARR, preoperative potassium level, and tumor size (Table 2). The median PAC in the *KCNJ5*-mutated group was higher than in the non–*KCNJ5*-mutated group but did not reach statistical

Table 2.   Clinical and Biochemical Phenotypes in Relation to *KCNJ5* Mutation Status

Variables	*KCNJ5*-Mutant Group (n = 67)	Nonmutant Group (n = 29)	*P* Value
Female, n (%)	43 (64)	16 (55)	0.407
Age, y[a]	47.48 ± 11.63	48.55 ± 13.15	0.705
BMI, kg/m^{2a}	26.02 ± 5.58	24.81 ± 5.35	0.380
Duration of hypertension, mo[b]	60 (24–120)	42 (24–120)	0.773
PAC, ng/dL[b]	54.90 (33.20–76.48)	34.70 (24.45–62.85)	0.058
PRA, ng/mL/h[b]	0.37 (0.20–0.67)	0.39 (0.19–0.62)	0.944
ARR[b]	125.23 (68.29–297.65)	104.14 (58.75–185.72)	0.311
Lowest potassium level, mmol/L[a] (3.6–5.0)	2.62 ± 0.55	2.40 ± 0.64	0.132
Preoperative potassium level, mmol/L[a] (3.6–5.0)	3.67 ± 0.58	3.58 ± 0.60	0.540
Daily potassium supplementation, mEq/d[b]	115 (60–120)	80 (40–110)	0.022
eGFR, mL/min/1.73 m^{2a}	85.95 ± 18.22	89.90 ± 25.85	0.508
Tumor size, cm[a]	1.77 ± 0.83	1.68 ± 0.85	0.652
Resolution of hypertension, n (%)	21 (31)	13 (68)	0.208
ZF-like, n (%)	58 (87)	17 (59)	0.002
ZG-like, n (%)	9 (13)	12 (41)	0.002

Abbreviation: BMI, body mass index.
[a]Data presented as mean ± SD.
[b]Data presented as median (IQR).

significance (54.90 vs 34.70 ng/dL; P = 0.058). A subgroup analysis on patients with p. Gly151Arg and p.Leu168Arg mutations groups did not show a statistically significant difference in the clinical characteristics.

C. Histologic Findings Correlate With KCNJ5 Mutation Status

In the *KCNJ5*-mutated group, 87% of adrenal tumors had histologic findings compatible with ZF-like cells, compared with 59% in the non–*KCNJ5*-mutated group (Table 3). There were significantly more ZF-like cells among patients with the *KCNJ5* mutation (P = 0.002).

D. Factors Associated With KCNJ5 Mutations

A univariate analysis was done to determine the clinical risk factors associated with the presence of the somatic *KCNJ5* mutation. PAC and the lowest potassium level were significantly associated with the mutation (P < 0.2 for both comparisons). These two factors were further evaluated in multivariate analysis. Only higher PAC was associated with the presence of the *KCNJ5* mutation (OR, 1.020; P = 0.039).

E. Effects of Adrenalectomy on Blood Pressure and Resolution of Hypertension

At 1 year postadrenalectomy or at the last visit of patients, 34 patients (35%) were able to discontinue all antihypertensive agents. Twenty-eight patients had hypertension resolution within 1 month after surgery. Antihypertensive agents were decreased or discontinued within 6 months after surgery. There was no significant difference in the resolution of hypertension between patients with different *KCNJ5* mutation status (P = 0.205). All patients with resolution of hypertension achieved resolution of hyperaldosteronism. Median PAC in these patients was 2.5 ng/dL (IQR, 1.40 to 3.75). Resolution of hyperaldosteronism was achieved in 90 patients (94%). After surgery, there were five patients who used more than two antihypertensive agents. Resolution of hypokalemia was achieved in all patients with preoperative hypokalemia.

F. Postoperative Hyperkalemia

Postoperative hyperkalemia occurred in 12 patients (13%). Permanent hyperkalemia occurred in five patients, one of whom required the treatment with fludrocortisone. Patients with postoperative hyperkalemia had a significantly longer duration of hypertension (120 vs 48 months; P = 0.022) and lower preoperative eGFR (64.70 vs 89.32 mL/min/1.73 m^2; P = 0.005) compared with patients without hyperkalemia. Preoperative mineralocorticoid antagonist use was not different among the two groups (2 vs 6; P = 0.310).

Table 3. Logistic Regression Models Predicting the Presence of *KCNJ5* Mutations in Patients with APAs

Clinical Risk Factors	Crude OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value
Age	0.993 (0.957–1.029)	0.687	—	—
Female	1.456 (0.600–3.521)	0.406	—	—
PAC	1.017 (1.000–1.034)	0.050[a]	1.020 (1.001–1.040)	0.039[b]
Lowest potassium level	1.923 (0.870–4.253)	0.106[a]	2.279 (0.932–5.573)	0.071
Tumor size	1.145 (0.644–2.037)	0.644	—	—

Abbreviation: OR, odds ratio.

[a]P value < 0.20 for univariate analysis.

[b]P value < 0.05 for multivariate analysis.

G. Postoperative Renal Function

Mean preoperative eGFR was 87.38 ± 20.89 mL/min/1.73 m^2. At 1 month after adrenalectomy, mean operative eGFR significantly decreased to 60.89 ± 40.66 mL/min/1.73 m^2 ($P < 0.001$). A significant decrease in mean eGFR was also observed at 12 months after adrenalectomy compared with the mean preoperative eGFR.

3. Discussion

The prevalence of somatic *KCNJ5* mutations in Thailand was 70% and was comparable with other Asian countries. Common somatic *KCNJ5* mutations were the substitutions p. Gly151Arg or p.Leu168Arg. The number of patients with mutations in p.Gly151Arg was greater than those with mutations in p.Leu168Arg. These findings were consistent with previous studies [3, 10, 15–21]. Studies by Monticone *et al.* [22] and Choi *et al.* [7] demonstrated a higher rate of mutations in p.Leu168Arg compared with p.Gly151Arg.

Because FFPE tissue was used in our study, we experienced DNA damage, which results in failure to amplify the template for PCR amplification in 24 patients (20%) of the total FFPE adrenal tissues [23]. However, the clinical characteristics of 24 patients who were excluded due to amplification failure were comparable to the 96 patients in this study in terms of age, PAC, potassium level, treatment of hypertension, and tumor size, as shown in Supplemental Table 1. We minimized sequence artifacts by selection and identification of the tumor-enriched area by a well-trained pathologist and carefully removed DNA crosslinks to obtain purity of DNA extraction. We also used specific primers for each strand of DNA to overcome DNA fragmentation from formalin-fixed tissue. Hong AR *et al.* [24] also demonstrated, using FFPE tissue, that the *KCNJ5* mutation rate in Korean patients with APA was 70%, whereas a study in New Zealand showed a lower frequency of *KCNJ5* mutations (38%) using either fresh-frozen or FFPE tissue [25].

The high prevalence of somatic *KCNJ5* mutations could have been associated with high sodium intake or could have been affected by the patient selection bias [6]. Because KCMH is a referral center, our patients with APA tended to present with apparent hypokalemia due to a later presentation of the disease. Our study showed that patients with somatic *KCNJ5* mutations required higher dosages of potassium supplement than those without mutations without a significant difference in preoperative potassium level. However, previous studies did not demonstrate the significant difference between potassium levels among two groups with different mutational status [6]. The amount of potassium supplementation might have been confounded by the level of compliance of the patients, which we did not address in this study. The proportion of patients with hypokalemia in this study was also high (98%) and was comparable with another study in Japan, which reported hypokalemia in 87% of patients [16]. Several studies in Europe showed less frequent occurrence of hypokalemia [20, 26]. It is possible that *KCNJ5* mutations are associated with higher proportions of hypokalemia in patients with APA.

The meta-analysis by Lenzini *et al.* [6] shows that patients with APA with somatic *KCNJ5* mutations have higher PAC and larger tumor size compared with those without somatic mutations. In our study, there was a trend toward greater PAC and adenoma size in patients with the mutations, but the sample size might have been too small to demonstrate the difference in these parameters. Unlike in the meta-analysis [6], the proportions of patients with somatic *KCNJ5* mutations of both sexes were not significantly different in this study (64% vs 55%; $P = 0.407$). Kitamoto *et al.* [27] and Wu *et al.* [10] reported similar results in Japanese and Taiwanese patients, respectively. In a multivariate analysis, higher PAC was associated with the presence of somatic *KCNJ5* mutations (OR, 1.017). Our study was not designed to assess the cutoff level for PAC to determine the presence of mutations.

Previous studies have shown that the percentage of patients with APA who had clinical success, defined as having normal blood pressure after adrenalectomy without the aid of antihypertensive medication, varied from 17% to 62% [28]. However, there is heterogeneity in the criteria used to classify the outcomes of adrenalectomy. In this study, 35% of patients had resolution of hypertension after adrenalectomy, but this number might not reflect the true

number of patients with resolution of hypertension due to the large number of dropouts within 1 year after surgery. Hypertension cure rates between the two mutation types of status were not different in this study. Wu *et al.* [10] demonstrated higher rates of resolution of hypertension in Taiwanese patients with somatic *KCNJ5* mutations. However, patients with somatic *KCNJ5* mutations in that study were younger and had shorter duration of hypertension compared with patients without the mutations. Two patients were excluded due to persistent hyper-aldosteronism, both of whom still had high plasma aldosterone concentration, suppressed renin, and hypokalemia after the operation. Neither of them underwent AVS before adrenalectomy. We then analyzed their clinical characteristics. There were no significant differences in terms of age, onset of hypertension, plasma aldosterone concentration, plasma renin activity, lowest serum potassium level, and tumor size among patients with persistent aldosteronism and the other group. DNA analysis was successfully done in one patient and showed no *KCNJ5* mutation. Germline mutation was not done in either patient.

The incidence of postoperative hyperkalemia in this study was 13%, which is similar to previous studies [26, 29]. The results of this study also showed that patients with longer duration of hypertension and lower preoperative eGFR experienced postoperative hyperkalemia more than patients with shorter duration of hypertension and better renal function. The result was consistent with the study by Park *et al.* [26], which determined the clinical risk factors associated with the occurrence of postoperative hyperkalemia. Patients with hypertension for >9.5 years had greater odds of developing postoperative hyperkalemia (OR, 10.50). The OR of an eGFR of <58.20 mL/min/1.73 m^2 was associated with an increased risk of postoperative hyperkalemia (OR, 26.6).

Postoperative renal function declined in patients with APAs after adrenalectomy, usually within the first month after surgery, and persisted for at least 12 months. This was compatible with a previous study in Japan [30].

There are some notable strengths of this study. This study established the prevalence of somatic *KCNJ5* mutations in Thai patients with APA. This confirms that the *KCNJ5* mutation rate is higher in Asia than in other continents, even in a neighboring country with a population comprised of different ethnic origins from China, Japan, and Korea. Another strength of this study is that we analyzed important clinical data of a large group of patients before and after surgery. A limitation in this study is due to its retrospective nature and the fact that other gene mutations in APAs were not evaluated.

There is no recommendation on the routine testing of somatic *KCNJ5* mutations in patients with APA. There is emerging evidence showing that macrolides could inhibit mutant *KCNJ5* K$^+$ channels from secreting excess aldosterone *in vitro* [31]. Patients with APA who are not surgical candidates could benefit from targeted therapy if future treatments specific to mutant potassium channels become available.

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