Genetic Characteristics of Aldosterone-Producing Adenomas in Blacks

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Abstract—Somatic mutations have been identified in aldosterone-producing adenomas (APAs) in genes that include *KCNJ5*, *ATP1A1*, *ATP2B3*, and *CACNA1D*. Based on independent studies, there appears to be racial differences in the prevalence of somatic *KCNJ5* mutations, particularly between East Asians and Europeans. Despite the high cardiovascular disease mortality of blacks, there have been no studies focusing on somatic mutations in APAs in this population. In the present study, we investigated genetic characteristics of APAs in blacks using a CYP11B2 (aldosterone synthase) immunohistochemistry-guided next-generation sequencing approach. The adrenal glands with adrenocortical adenomas from 79 black patients with primary aldosteronism were studied. Seventy-three tumors from 69 adrenal glands were confirmed to be APAs by CYP11B2 immunohistochemistry. Sixty-five of 73 APAs (89%) had somatic mutations in aldosterone-driver genes. Somatic *CACNA1D* mutations were the most prevalent genetic alteration (42%), followed by *KCNJ5* (34%), *ATP1A1* (8%), and *ATP2B3* mutations (4%). *CACNA1D* mutations were more often observed in APAs from males than those from females (55% versus 29%, P=0.033), whereas *KCNJ5* mutations were more prevalent in APAs from females compared with those from males (57% versus 13%, P<0.001). No somatic mutations in aldosterone-driver genes were identified in tumors without CYP11B2 expression. In conclusion, 89% of APAs in blacks harbor aldosterone-driving mutations, and unlike Europeans and East Asians, the most frequently mutated aldosterone-driver gene was *CACNA1D*. Determination of racial differences in the prevalence of aldosterone-driver gene mutations may facilitate the development of personalized medicines for patients with primary aldosteronism. **(*Hypertension*. 2019;73:885-892. DOI: 10.1161/HYPERTENSIONAHA.118.12070.)** ● **Online Data Supplement**

Key Words: adrenocortical adenoma ■ adrenal glands ■ aldosterone ■ hyperaldosteronism ■ mutation

The identification of somatic and germline mutations in patients with primary aldosteronism has provided insights into the mechanisms causing the dysregulation of adrenal aldosterone production. Mutations in genes that include potassium voltage-gated channel subfamily J member 5 (*KCNJ5*),[1] ATPase, Na^+/K^+ transporting, α1-polypeptide (*ATP1A1*) and ATPase, Ca^{2+} transporting, plasma membrane 3 (*ATP2B3*),[2] L-type voltage-gated calcium channel subunit alpha-1D (*CACNA1D*),[3,4] T-type voltage gated-calcium channel subunit alpha 1H (*CACNA1H*),[5] and chloride voltage-gated channel 2 (*CLCN2*)[6,7] have been found in aldosterone-producing adenomas (APAs) and familial hyperaldosteronism. Most of the mutations are considered to activate the intracellular signaling pathway that normally regulates aldosterone production. Numerous studies have been conducted to determine the prevalence of somatic mutations in APAs[8–14] and the *KCNJ5* gene is thought to be the most frequently mutated gene in APAs.[15] Interestingly, there appear to be racial differences in the prevalence of somatic mutations. Most striking are the findings that *KCNJ5* somatic mutations are much more common in East Asians when compared with Europeans.[10,12–16] Despite the growing evidence of racial differences in the somatic mutations spectrum in APAs, there have been no studies focusing on

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Americans of African descent (blacks). Importantly, in the United States, blacks are known to have poorer overall cardiovascular health and higher cardiovascular disease mortality than Americans of European descent (whites).[17] Hypertension is a significant risk factor for developing cardiovascular disease morbidity and mortality in blacks, and race-specific treatment for hypertension is recommended by current clinical practice guidelines, ie, calcium channel blocker or diuretics as an initial therapy for hypertensive black patients.[18,19] Regarding the association of aldosterone with hypertension, blacks appear to be particularly vulnerable to the effects of excess aldosterone production.[20–22]

We recently demonstrated that a sequencing approach targeting the entire coding region of genes mutated in APAs based on the tumor expression of CYP11B2 (aldosterone synthase), required for final steps of aldosterone biosynthesis and known to be highly expressed in APAs, provides more accurate determination of APA-related somatic mutations than non CYP11B2-directed, conventional mutation hotspot sequencing.[23] In this multicenter collaborative study, using this state-of-the-art technique, we investigated the prevalence of somatic mutations in APAs in a cohort of black patients with a hypothesis that blacks have a unique APA-related somatic mutation spectrum.

Materials and Methods

The authors declare that all supporting data are available within the article and its online-only Data Supplement.

Patients

The study included 79 black patients with primary aldosteronism who underwent unilateral adrenalectomy at the University of Michigan, National Institutes of Health, medical centers within the Southern California Permanente Medical Group, University of Pennsylvania, Vanderbilt University Medical Center, and Brigham and Women's Hospital. Race was determined by self-identification as black or African American. The diagnosis of primary aldosteronism was made based on institutional consensus available at the time or the Endocrine Society clinical practice guideline.[24] The results of cross-sectional imaging and adrenal venous sampling were used for subtype classification. The patients were selected based on the availability of archival adrenal tumor formalin-fixed paraffin-embedded blocks. Formalin-fixed paraffin-embedded tumor sections were used for immunohistochemistry and genetic analysis. This study was approved by Institutional Review Boards at each participating center.

Targeted Next-Generation Sequencing

Genomic DNA of APAs, CYP11B2-negative adrenocortical tumors, and CYP11B2-expressing cell foci in adjacent adrenals (aldosterone-producing cell clusters [APCCs][25]) were isolated separately from formalin-fixed paraffin-embedded sections using AllPrep DNA/RNA FFPE kit (QIAGEN) as described previously.[26] Multiplexed PCR based targeted next-generation sequencing (NGS) was performed using Ion Torrent Ampliseq sequencing (Thermo Fisher Scientific). The detailed method of NGS was described previously.[27] The panel for library preparation included the full coding regions of APA-related genes (*KCNJ5*, *ATP1A1*, *ATP2B3*, and *CACNA1D*). In the panel, genes that are associated with other adrenocortical diseases (protein kinase, cAMP-dependent, catalytic, α [*PRKACA*], protein kinase, cAMP-dependent, regulatory, type I, α [*PRKAR1A*], and armadillo repeat containing 5 [*ARMC5*]) and oncogene hotspots in guanine nucleotide-binding protein subunit α (*GNAS*) and β-catenin (*CTNNB1*) were also included. Methods for somatic variant identification are described in the online-only Data Supplement.

Statistical Analysis

Clinical data are presented as medians with interquartile ranges and counts and frequencies for categorical variables. For comparison, Mann-Whitney *U* test and Fisher Exact test were performed unless otherwise indicated. The SigmaPlot 12.4 software package was used for the statistical analysis (Systat Software, Inc). A P value of <0.05 was considered significant.

Additional detailed descriptions of materials and methods are available in the online-only Data Supplement.

Results

Histopathologic Characteristics of Adrenocortical Tumors From Blacks With Primary Aldosteronism

Of 79 adrenals with adrenocortical tumors, 69 (87%) had at least 1 CYP11B2-expressing tumor that was considered an APA by CYP11B2 immunohistochemistry (Figure 1). Four out of 69 adrenals had 2 independent APAs within the same adrenal, resulting in a total of 73 APAs. In ten adrenals containing APAs, CYP11B2-negative adrenocortical tumors were also observed. As an example of multiple tumors within the same adrenal gland, histopathologic findings of an adrenal containing 2 independent APAs and 1 CYP11B2-negative adrenal tumor are shown in Figure 2. Of 10 adrenals with non-APA, 9 (90%) had APCCs (CYP11B2-expressing cell foci just beneath the capsule of adjacent adrenal[25]). Some of the APCCs observed in our cohort appeared to be much larger in size compared with those found in normal adrenal glands. Representative histopathologic findings of an adrenal with non-APA (a CYP11B2-negative adrenal tumor with multiple APCCs) are shown in Figure 3. By immunohistochemistry, the expression of CYP11B1 (11β-hydroxylase), that is required for cortisol production, was highly expressed in the CYP11B2-negative tumor, whereas it was mostly negative or weakly expressed in APCCs.

Clinical characteristics of the study population are summarized in Table 1. When comparing the clinical characteristics between patients with APA and those with non-APA, median plasma aldosterone concentration was significantly higher in patients with APA than those with non-APA (38.8 versus 21.4 ng/dL; P=0.020). There was no significant difference in the other clinical parameters between the 2 groups.

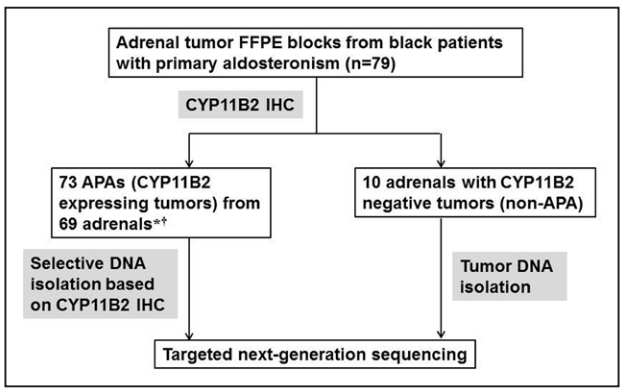

Figure 1. Study design based on the results of CYP11B2 immunohistochemistry. CYP11B2-expressing tumors were considered as aldosterone-producing adenomas (APAs) by CYP11B2 immunohistochemistry (IHC). *Four adrenals had 2 independent APAs within the same adrenal; †Ten CYP11B2-negative tumors adjacent to APAs were also assessed. FFPE, formalin-fixed paraffin-embedded.

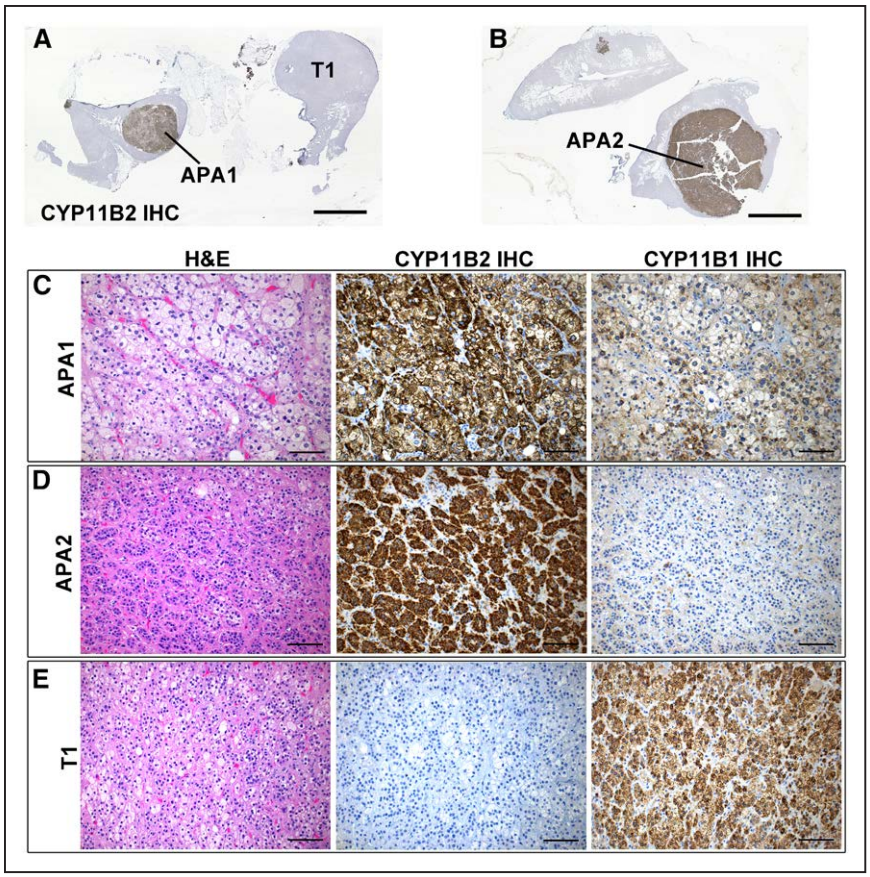

Figure 2. Histopathologic findings of multiple aldosterone-producing adenomas (APAs) and a CYP11B2-negative adrenocortical adenoma within the same adrenal gland. **A** and **B**, Scanned images of adrenal gland and tumors following CYP11B2 immunohistochemistry (IHC; brown, CYP11B2). Two FFPE (formalin-fixed paraffin-embedded) blocks were used for examination (**A**, block 1; and **B**, block 2). Scale bar, 5 mm. **C**–**E**, High magnification view of each tumor (**C**, APA1; **D**, APA2; and **E**, T1). APA1 is mostly composed of lipid-rich clear cells with moderate expression of CYP11B1 as well as high CYP11B2, whereas APA2 is mainly composed of lipid-poor compact cells with intense CYP11B2 expression. Scale bar, 100 μm. H&E indicates hematoxylin and eosin staining; and T1, CYP11B2-negative tumor.

Aldosterone-Driver Gene Mutations in APAs From Blacks

A total of 73 APAs from 69 black patients were studied to determine the somatic mutation spectrum. Somatic mutations identified by targeted NGS are summarized in Table 2. Unlike European and East Asian cohorts, the most prevalent aldosterone-driver gene alterations in blacks were seen in *CACNA1D* (n=31, 42%), followed by *KCNJ5* (n=25, 34%), *ATP1A1* (n=6, 8%), and *ATP2B3* (n=3, 4%). The distribution of APA-related somatic mutations in each participating center is shown in Table S1 in the online-only Data Supplement and Table S2. In our cohort, *KCNJ5* mutations were more often observed in APAs from females than those from males (57% versus 13% in APAs from males; $P<0.001$), whereas *CACNA1D* mutations were more frequently seen in APAs from males compared with those from females (55% versus 29% in APAs from females; $P=0.033$). A comparison of genotype with basic clinical characteristics and post-surgical outcome are summarized in Table S3 and Table S4, respectively.

Importantly, the sequencing approach used in the present study is different from the method that has been used in most of the previous studies (non CYP11B2 immunohistochemistry-directed, hotspot sequencing approach). We, therefore, assessed racial differences in somatic mutation prevalence using the data from our recent study focusing on whites in which the CYP11B2 immunohistochemistry-guided NGS approach was used.[23] The somatic mutation distribution in blacks and whites is shown in Figure S1. When comparing the prevalence of somatic *CACNA1D* mutations with APAs from whites, APAs from blacks had a significantly higher frequency of *CACNA1D* mutations (42% versus 21% in whites; $P=0.01$ by χ^2 test).

Of the variants determined by NGS, to our knowledge, 8 were previously unreported (6 in *CACNA1D*, 1 in *KCNJ5*, and 1 in *ATP1A1*, Table 2). These variants were confirmed to be somatic because there was no evidence of the variants in adjacent normal adrenal tissue. NGS results of the previously unreported mutations are summarized in Table S5. Notably, of the novel mutations, the *CACNA1D* p.R993T (c.G2978C) mutation was recurrently identified in 3 APAs (2 males and 1 female).

One APA harbored concomitant somatic mutations in *CACNA1D* (p.G403R) and *CTNNB1* (p.G34R) with similar variant allele frequencies (39% and 38%, respectively). No evidence of distinct heterogeneity in CYP11B2 expression was observed in the tumor (Figure S2), supporting the possibility of coexistence of *CACNA1D* and *CTNNB1* mutations within a single APA. Of 4 adrenals with multiple APAs, one had 2 APAs with independent *KCNJ5* (p.L168R, APA1 in Figure 2A and 2C) and *ATP1A1* (p.I955_E960delinsK, APA2 in Figure 2B and 2D) somatic mutations, one had 2 APAs with independent *CACNA1D* (p.F747V) and *ATP1A1* (p.L104R) somatic mutations as described previously,[26] and there was 1 adrenal with 2 independent APAs both harboring the same *KCNJ5* p.L168R mutation. The remaining adrenal had 2 APAs, and a *CACNA1D* (p.S652L) mutation was identified in 1 APA, and no mutation was found in the other.

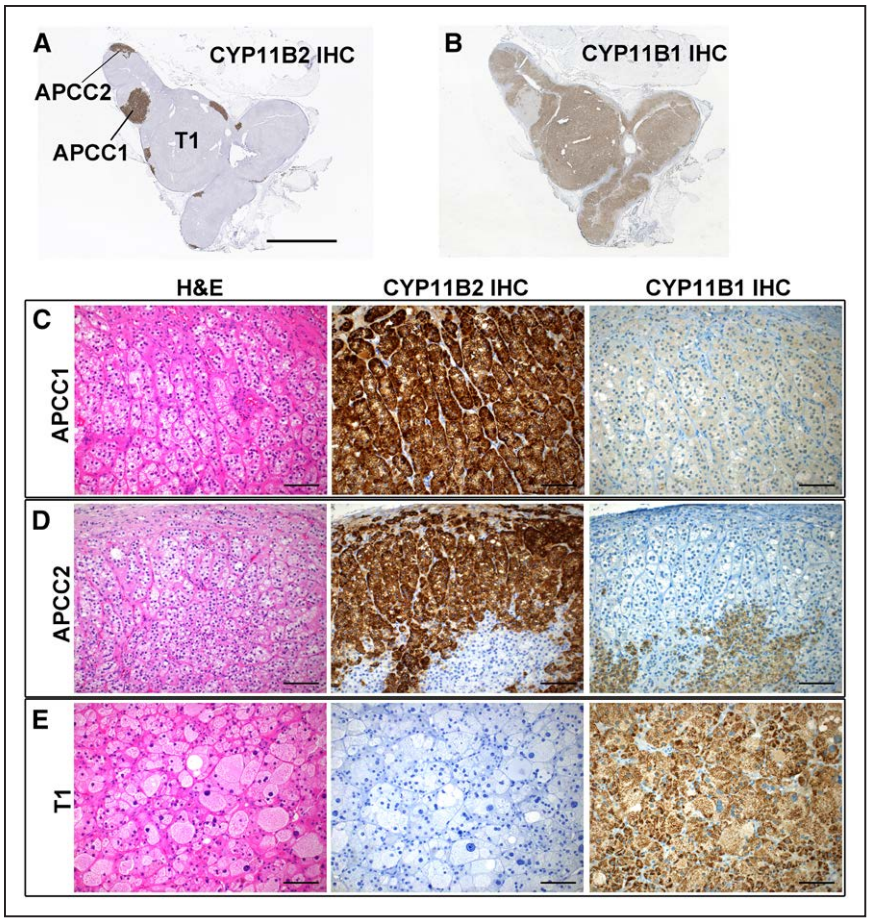

Figure 3. Histopathologic findings of multiple aldosterone-producing cell clusters and a CYP11B2-negative adrenocortical adenoma. **A** and **B**, Scanned images of adrenal gland following immunohistochemistry (IHC) for CYP11B2 (**A**) and CYP11B1 (**B**); Scale bar, 5 mm. **C**–**E**, Higher magnification view of aldosterone-producing cell cluster (APCC1) (**C**), APCC 2 (**D**), and the CYP11B2-negative tumor (T1; **E**). Scale bar, 100 µm. H&E indicates hematoxylin and eosin staining.

Somatic Mutations in CYP11B2-Negative Adrenal Tumors and APCCs

No APAs were observed in 10 adrenals (non-APA) from 10 black patients with primary aldosteronism by CYP11B2 immunohistochemistry (Figure 1). We performed targeted NGS on these 10 CYP11B2-negative adrenal tumors as well as 10 CYP11B2-negative adrenal tumors adjacent to APAs. For sequencing analysis, we further included 8 APCCs adjacent to adrenal tumors from 7 patients, 1 with an APA and 6 with non-APA. Of 20 CYP11B2-negative tumors assessed, 2 harbored the *PRKACA* hotspot mutation (p.L206R) and 1 had an activating *GNAS* mutation (p.R201C, T1 in Figure 3, Table S6). Both mutations have previously been identified in cortisol-producing adenomas.[28,29] No aldosterone-driving gene mutations were found in any of the CYP11B2-negative adrenal tumors. On the contrary, somatic mutations in aldosterone-driving genes were observed in 7 out of 8 APCCs (Table S7). Of the mutations identified in APCCs, all but 1 (*CACNA1D* p.I1015S, c.T3044G) were previously reported in APA,[2,4,11] supporting the concept that APCCs contribute to renin-independent aldosterone production. Of note, the *CACNA1D* p.I1015S mutation was also identified in 1 APA in our cohort.

Discussion

In this multicenter collaborative study, we investigated the somatic mutation spectrum of APAs in blacks with primary aldosteronism using a CYP11B2 immunohistochemistry-guided gene targeted NGS approach. Interestingly, *CACNA1D* mutations were the most prevalent genetic alteration in APAs from blacks (42%), whereas the *KCNJ5* gene is most frequently mutated in whites,[23] European,[11,30] and East Asian populations.[12,13] Somatic mutations in *CACNA1D* have been considered a rare event with the approximate prevalence of 9% in Europeans[11] and <2% in East Asians.[12–14] The *CACNA1D* gene encodes $Ca_v1.3$ (α1 subunit of voltage-dependent L-type calcium channel). $Ca_v1.3$ contains 4 homologous repeats (I-IV), and each has 6 transmembrane segments (S1-S6). $Ca_v1.3$ is expressed in human adrenal zona glomerulosa where physiological aldosterone biosynthesis occurs.[3,31] Cell-based studies demonstrated that activating mutations in the *CACNA1D* gene can cause increased intracellular Ca^{2+} influx, resulting in enhanced aldosterone production[3,31] and inhibitory effect of nifedipine on aldosterone production from H295R cells with mutant $Ca_v1.3$ was also observed.[31] In the present study, 6 previously unreported somatic *CACNA1D* mutations were identified. These mutations are located in the regions encoding transmembrane S4 segment serving as the voltage-sensor (p.R619P, p.R990G, and p.R993T), cytoplasmic S4-S5 linker (p.C1007R and p.I1015S), and extracellular domain (p.V309A). The *CACNA1D* p.R990G mutation was found in the same residue where a somatic mutation (p.R990H) was previously identified in sporadic APA.[4] Importantly, the *CACNA1D* p.R993T was recurrently observed in 3 independent APAs in our cohort.

In our cohort, the *KCNJ5* gene was the second most frequently altered gene. As seen in Europeans[11] and whites,[23]

Table 1. Clinical Characteristics of Studied Subjects

Characteristics	APA	Non-APA
N	69	10
Age, y	52 (43–59)	55 (50–60)
Male (n/%)	36/52%	5/50%
Female (n/%)	33/48%	5/50%
Systolic blood pressure (mm Hg)	144 (133–159)	149 (138–171)
Diastolic blood pressure (mm Hg)	90 (80–98)	88 (82–98)
Number of anti-hypertensive medications	3.0 (2.0–4.0)	3.5 (3.0–5.3)
Prevalence of hypokalemia (N/%)	62/90%	9/90%
PAC (ng/dL)	38.8* (26.1–55.7)	21.4 (18.8–34.9)
PRA (ng/mL/h)†	0.2 (0.1–0.6)	0.2 (0.2–0.5)

Data are expressed as medians with interquartile ranges for continuous variables and counts and frequencies for categorical variables. The classification and definition of APA and non-APA are shown in Figure 1. Hypokalemia was defined as a serum potassium concentration <3.5 mEq/L or if potassium supplementation was indicated. PAC indicates plasma aldosterone concentration; and PRA, plasma renin activity.

*$P<0.05$ vs non-APA.

†Data from 2 patients (1 APA and 1 non-APA) were not available.

Table 2. Somatic Mutations Identified in APAs From Blacks

Somatic Mutations	APA From Men (n=38)	APA From Women (n=35)	Total (n=73)
CACNA1D gene	21 (55%)	10 (29%)	31 (42%)
p.V309A*	1	0	1
p.V401L	0	1	1
p.G403R	2†	1	3
p.G403R (exon 8B)	6	0	6
p.R619P*	1	0	1
p.S652L	0	1	1
p.F747V	3	0	3
p.F747L	0	1	1
p.F747C	0	1	1
p.I750F	1	0	1
p.I750M	1	0	1
p.R990G*	1	0	1
p.R993T*	2	1	3
p.A998V	2	2	4
p.C1007R*	0	1	1
p.I1015S*	1	0	1
p.V1151F	0	1‡	1
KCNJ5 gene	5 (13%)	20 (57%)	25 (34%)
p.[T148I;T149S]	0	1	1
p.T149delinsTT	1	0	1
p.T149delinsMA*	1	0	1
p.G151R	2	11	13
p.L168R	1	8	9
ATP1A1 gene	5 (13%)	1 (3%)	6 (8%)
p.L104R	4	1	5
p.I955_E960delinsK*	1	0	1
ATP2B3 gene	2 (5%)	1 (3%)	3 (4%)
p.V424_L425del	2	1	3
Mutation negative	5 (13%)	3 (9%)	8 (11%)

Reference sequences used for determining amino acid changes: NM_000890 for KCNJ5, NM_000701 for ATP1A1, NM_021949 for ATP2B3, NM_001128839 and NM_000720 (exon 8B) for CACNA1D, and NM_001904 for CTNNB1.

*Previously unreported in APAs.

†Concomitant CTNNB1 (p.G34R) mutation was observed in 1 APA.

‡Confirmed by the replicate library because of low sample quality.

KCNJ5 mutation was more often observed in black females than males. Interestingly, this sex difference in *KCNJ5* mutation has not been obvious in East Asian countries, including Japan and China, where the majority of APAs have *KCNJ5* somatic mutations.[10,12,14] In the present study, a novel *KCNJ5* somatic mutation (p.T149delinsMA) was also identified. The *KCNJ5* gene encodes the GIRK4 (G-protein-activated inwardly rectifying potassium channel 4) that is highly expressed in the zona glomerulosa of human adrenal cortex.[1] The position 149 locates near the selectivity filter of its ion channel pore, and several somatic mutations involving this residue have been reported.[13,14,23,32,33] Finally, a novel somatic mutation in the *ATP1A1* gene was found in the present study. The *ATP1A1* gene encodes α1 -subunit of the Na^+/K^+ ATPase. In vitro studies demonstrated that *ATP1A1* mutations lead to cell membrane depolarization, resulting in increased *CYP11B2* transcription, and inappropriate aldosterone production.[2,34] Although the *ATP1A1* p.I955_E960delinsK mutation has not been previously reported, several mutations locating in the same TM9 (transmembrane domain) have been documented.[4,23,30] Functional characterization of these previously unreported mutations will be required in the future studies. Whole exome sequencing of mutation negative APAs will also be useful to determine novel gene mutations that may contribute to autonomous aldosterone production.

Activating mutations in exon 3 of the *CTNNB1* gene that encodes β-catenin have been identified in a subset of APAs[16,35,36] as well as other adrenocortical adenomas and adrenocortical carcinomas.[37] Although *CTNNB1* mutations in APAs have been thought to be mutually exclusive to aldosterone-driver genes including *KCNJ5*, *ATP1A1*, *ATP2B3*, and *CACNA1D*,[35] one of our APA had concomitant *CACNA1D* and *CTNNB1* mutations, indicating the possible role of activating *CTNNB1* mutations for tumor development rather than that as an aldosterone stimulating factor. To determine the significance of *CTNNB1* mutations in APAs, further investigations will be needed.

An important observation in the present study is genetic characterization of CYP11B2-negative adrenal tumors and APCCs. In accordance with previous studies,[23,26,38] no aldosterone-driver gene mutation was identified in any of the CYP11B2-negative adrenal tumors. This finding raises a concern regarding the use of adrenal-sparing surgery in patients with primary aldosteronism. Although adrenal-sparing surgery

is beneficial for rare cases such as those with bilateral APAs, careful consideration is required for the application of this surgical procedure with the guidance of segmental adrenal venous sampling.[39] Interestingly, somatic mutations in *PRKACA* (p.L206R) and *GNAS* (p.R201C) were identified in 2 and 1 CYP11B2-negative adrenal tumor, respectively. Recently, Rhayem et al[40] reported the *PRKACA* mutation as a rare finding in APA. They sequenced 122 APAs, and somatic mutations were found only in 2 of them; p.H88D and p.L206R. Functional analysis revealed reduced enzymatic PKA activity in the p.H88D mutation, whereas constitutive activity was seen in p.L206R mutation[40] which has been frequently observed in cortisol-producing adenomas causing overt Cushing syndrome.[28,29] Further, the adenoma with *PRKACA* p.L206R mutation was predominantly positive for CYP11B1, whereas CYP11B2 expression was observed only in few adenoma cells and the patient was biochemically confirmed as having hypercortisolism.[40] Of the 2 cases with *PRKACA* p.L206R mutation in our black cohort, clinically, one had concomitant primary aldosteronism and overt Cushing syndrome (reported previously from our group[33]). The other patient had obesity with type 2 diabetes mellitus and showed incomplete suppression of serum cortisol (2.5 µg/dL) after a 1 mg dexamethasone suppression test. A recent study reported that somatic *GNAS* mutation (p.R201C) was identified in 2 out of 33 APAs although tumor CYP11B2 expression (indicating the capacity to produce aldosterone) was not assessed.[41] Of note, both patients with *GNAS*-mutated tumors in the study had autonomous cortisol secretion as well as primary aldosteronism.[41] The role of *GNAS* mutations on pathogenesis of primary aldosteronism is still unclear, and further study is needed.

To better understanding the cause of inappropriate aldosterone production in adrenals from patients with primary aldosteronism, we investigated somatic mutations in APCCs in adjacent adrenals. Of 8 APCCs, 7 had somatic mutations in *CACNA1D* or *ATP1A1* gene in agreement with our previous observations of somatic mutations in APCCs in American and Japanese cohorts[27,42] as well as findings in adrenals from patients with idiopathic hyperaldosteronism.[43] Notably, most of the mutations identified in our study (7/8, 88%) were previously reported in APAs and 4 of them (*CACNA1D* p.G403R and p.I750M, *ATP1A1* p.L104R, and p.V332G) have been functionally characterized,[2–4,34] suggesting the potential contribution of APCCs to autonomous aldosterone production in patients with primary aldosteronism.

There are several limitations in this study. First, the newly identified somatic mutations in this study were not functionally characterized. In vitro studies will be needed to determine the effect of these variants on excess aldosterone production from mutant-expressing cells. Second, the retrospective study design has the potential for selection bias. To avoid sample selection bias, a dedicated larger prospective study will be required for more accurate assessment of somatic mutation spectrum. Third, because of the small sample sizes of the *ATP1A1*, *ATP2B3*, and mutation-negative groups, the genotype-phenotype relationship was not statistically analyzed. A similar larger prospective study using the outcome classification system developed by the PASO (Primary Aldosteronism Surgery Outcome) investigators[44] will provide better assessment of the genotype and clinical phenotype, including post-surgical outcome. Lastly, since our study focuses on black populations, we were not able to determine racial and genotype-phenotype correlation. Further multi-racial comprehensive study will be needed.

Perspectives

Cardiovascular health in blacks has been an important issue because of the high incidence of hypertension and high cardiovascular disease mortality. Primary aldosteronism is a common cause of hypertension and enhances cardiovascular disease mortality. To date, the genetic causes of APAs from blacks have not been defined. Somatic *CACNA1D* mutations appear to be the major genetic cause of inappropriate aldosterone production in APAs, especially in black males. The results of mutation prevalence suggest that black males with primary aldosteronism might benefit from L-type calcium channel blockers. Further in vitro and clinical studies will be needed to assess the efficacy of calcium channel blockers as a therapy for blacks with primary aldosteronism because of somatic *CACNA1D* mutations.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Aldosterone synthase immunohistochemistry-guided next-generation sequencing revealed that somatic mutations in the *CACNA1D* gene, encoding voltage-dependent L-type calcium channel subunit alpha-1D, were the most common genetic causes of aldosterone-producing adenomas in blacks.

What Is Relevant?

- Our study provides a better understanding of the molecular characteristics of aldosterone-producing adenomas in blacks and indicates the importance of defining racial differences in somatic mutation prevalence in aldosterone-producing adenomas.

Summary

This study demonstrates that the *CACNA1D* is the most frequently altered gene in aldosterone-producing adenomas in blacks. Determination of the race-specific somatic mutation spectrum may provide the foundation for future research on personalized diagnostics and therapeutics for patients with primary aldosteronism.