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Novel Somatic Mutations in Primary Hyperaldosteronism are related to the Clinical, Radiological and Pathological Phenotype

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Summary

Aldosterone-producing adenomas (APAs) and bilateral adrenal hyperplasia are important causes of secondary hypertension. Somatic mutations in *KCNJ5*, *CACNA1D*, *ATP1A1*, *ATP2B3* and *CTNNB1* have been described in APAs.

Objective—To characterize clinical-pathological features in APAs and unilateral adrenal hyperplasia, and correlate them with genotypes.

Design—Retrospective study.

Subjects and Measurements—Clinical and pathological characteristics of 90 APAs and 7 diffusely or focally hyperplastic adrenal glands were reviewed, and samples were examined for mutations in known disease genes by Sanger or exome sequencing.

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Nothing to declare.

Results—Mutation frequencies were: *KCNJ5*, 37.1%; *CACNA1D*, 10.3%; *ATP1A1*, 8.2%; *ATP2B3*, 3.1%; *CTNNB1*, 2.1%. Previously unidentified mutations included I157K, F154C and 2 insertions (I150_G151insM and I144_E145insAI) in *KCNJ5*, all close to the selectivity filter, V426G_V427Q_A428_L433del in *ATP2B3*, and A39Efs*3 in *CTNNB1*. Mutations in *KCNJ5* were associated with female, and other mutations with male gender ($p=0.007$). On computed tomography, *KCNJ5*-mutant tumors displayed significantly greater diameter ($p=0.023$), calculated area ($p=0.002$) and lower pre-contrast Hounsfield Units ($p=0.0002$) vs. tumors with mutations in other genes. Accordingly, *KCNJ5*-mutant tumors were predominantly comprised of lipid-rich fasciculata-like clear cells, whereas other tumors were heterogeneous ($p=5\times 10^{-6}$ vs. non-*KCNJ5* mutant and $p=0.0003$ vs. wild type tumors, respectively). *CACNA1D* mutations were present in two samples with hyperplasia without adenoma.

Conclusions—*KCNJ5* mutant tumors appear to be associated with fasciculata-like clear cell predominant histology and tend to be larger with a characteristic imaging phenotype. Novel somatic *KCNJ5* variants likely cause adenomas by loss of potassium selectivity, similar to previously described mutations.

Keywords

KCNJ5; *CACNA1D*; ATPase; primary aldosteronism; computed tomography

Introduction

Primary hyperaldosteronism (PH) features overproduction of aldosterone despite suppressed plasma renin activity (PRA). The resulting Na^+ retention produces hypertension, and elevated K^+ excretion may cause hypokalemia. The prevalence of PH is 6-11% in referral centers and increases with the severity of hypertension.¹⁻³ Higher rates of cardiovascular disease in these patients warrant early diagnosis and therapy.⁴ About one third of patients with PH have aldosterone producing adrenocortical adenoma (APA), a small subset (~2%) have unilateral hyperplasia, and most of the remainder exhibit idiopathic hyperaldosteronism (IHA, also known as bilateral adrenal hyperplasia).⁵ Patients with unilateral disease are critical to identify since unilateral adrenalectomy leads to improvement in blood pressure and serum K^+ concentrations in nearly all cases, and remission of hypertension is observed in 40-50%.^{2, 6-9} In contrast, patients with bilateral disease are best managed medically.²

Two somatic mutations in the K^+ channel *KCNJ5* (G151R and L168R) cause ~40% of APAs in large cohorts, possibly with higher frequencies in Asian cohorts.¹⁰⁻¹⁹ Mutations affect K^+ selectivity, leading to Na^+ influx, cellular depolarization and activation of voltage-gated Ca^{2+} channels, resulting in Ca^{2+} influx. Increased intracellular Ca^{2+} is the sufficient signal for aldosterone production in normal adrenal glomerulosa²⁰, and chronic stimulation produces cell proliferation. Accordingly, expression of mutant *KCNJ5* channels in a human adrenal carcinoma cell line increases intracellular calcium and aldosterone production.²¹

Additional genes are less frequently mutated than *KCNJ5* and appear to phenocopy the effect on intracellular Ca^{2+} concentration. Gain-of-function *CACNA1D* (voltage-gated Ca^{2+} channel $\text{Ca}_v1.3$) mutations are present in 5-11% of APAs^{15, 22, 23} and cause increased calcium influx through the mutant channel by shifting the voltage dependence of activation

to less depolarized potentials and, in some cases, impairing inactivation. Mutations in two members of the P-type ATPase gene family (*ATP1A1* or *ATP2B3*) collectively occur in ~7% of the cases.^{15, 23-25} *ATP1A1* mutations lead to permeability of the pump for Na⁺ or H⁺ ions in a channel-like mode, again causing depolarization.²³ *CTNNB1* (β-catenin) mutations are rare and also occur in cortisol-secreting or non-functional adenomas. Interestingly, constitutive activation of β-catenin in mice leads to adrenal hyperplasia, the development of malignant characteristics and primary aldosteronism.^{22-24, 26, 27}

The finding of mutations in either of five genes in APAs raises the question whether mutations in specific genes are associated with particular histological or clinical findings, and whether similar mutations are present in diffuse hyperplasia. We here report the mutational status, radiographic findings on computed tomography (CT) and histopathology of a cohort of 90 APAs and 7 glands with diffuse or focal hyperplasia from 3 centers in the USA and Germany.

Material and Methods

Clinical characterization, Radiography & Tissues

The study was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained prior to adrenalectomy, and the study was approved by institutional review boards at Yale, Düsseldorf and Bonn Universities. Formalin-fixed paraffin embedded (FFPE) tissue samples of 67 patients with sporadic PH who had unilateral adrenalectomy at Yale New Haven Hospital, 19 from Düsseldorf and 11 from Bonn (tumor size > 1 cm) were analyzed. The mutational status of 35 of these cases has been previously reported.^{10, 22} Plasma aldosterone concentration and PRA (Yale, Bonn) or plasma renin concentration (PRC, Düsseldorf) were measured. An aldosterone to renin ratio (ARR) >20 ng/dl:ng/ml/h, >50 pg/ml:μIU/mL or >50 pg/ml:pg/ml was considered indicative of PH.^{2, 28} In 22 cases, archived pathology material was used for mutational analysis but the original diagnostic biochemistry was not available. In these cases, careful review of the patients' charts and clinical correspondence was taken to provide sufficiently robust evidence that the diagnosis of PA was correct. The diagnosis was confirmed by Na-Cl infusion, oral Na⁺ loading and/or adrenal venous sampling (AVS, cortisol [adrenal]/cortisol [periphery] >5, lateralization confirmed with dominant to non-dominant ratio >4 at Yale (with ACTH infusion), cortisol [adrenal]/cortisol [periphery] >2, lateralization confirmed with dominant to non-dominant ratio 3 in Düsseldorf (without ACTH infusion); AVS was not systematically performed in Bonn). Due to technical problems, especially on the right side, AVS could not be performed accurately in all patients.²⁹ When unilateral aldosterone hypersecretion was diagnosed, unilateral adrenalectomy was performed. Düsseldorf cases were ascertained through the German Conn's registry using published procedures³⁰; all cases showed lateralization in AVS.

Pre-operative CT images were retrospectively analyzed in 58 available cases. Each CT was independently re-assessed by an experienced endocrine radiologist (A.M.) who was blinded to tumors' mutation status. Tumor measurements were assessed in two dimensions,³¹ and Hounsfield units were averaged over the area of the tumor. Biochemical and clinical follow-

up was typically performed immediately, at 1-2 weeks, 6-12 weeks and 6-12 months after surgery.

Specimens were reviewed by two pathologists blinded to genotypes and categorized by gross assessment (size, number of nodules). Non-tumor cortex was assessed for the presence of hyperplasia (extensive micronodules or simple cortical expansion). Tumor histology was recorded as percent of zona fasciculata-like clear cells and categorized as containing 33%, 34-50%, 51%-65% or 66% clear cells. Variations in nuclear size and shape were graded as 0 (absent), 1+ (mild), 2+ (moderate) and 3+ (severe). The highest score was recorded, even if changes were focal.

DNA isolation and sequencing

1-3 mm cores (depending on the size of the nodule) or sections of FFPE tissue from nodules and normal adjacent adrenocortical tissue were obtained. For Yale and Düsseldorf samples, genomic DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen). For Bonn samples, cores were deparaffinized in limonene (2× 30 min, 1,000 rpm, 60°C), re-hydrated, air-dried, incubated at 60°C (8 h) in lysis buffer (50 mM Tris-HCl, pH 8.4, 1 mM EDTA, 0.5% Tween-20, 2 mg/ml Proteinase K) and homogenized by pipetting. Samples were incubated with 20 µL Proteinase K (20 mg/ml; 16 h, 60°C, 1,000 rpm). Lysates were mixed with binding buffer (6 M guanidiniumthiocyanate, 0.1 M Tris, pH 7.5) and ethanol. DNA was purified using a NucleoSpin Extract II spin column (Macherey&Nagel).

For select samples, exome sequencing was performed as previously described (Table 1).²²

Genomic DNA was subjected to amplification by PCR using previously published primer sets targeting *KCNJ5*, *ATP1A1*, *ATP2B3*, *CTNNB1* and *CACNA1D*, followed by bidirectional sequencing.²² Samples without identified mutations were amplified and sequenced using additional primers for the *CACNA1D* gene: 1D_V259D_F: 5'-CCTTCCTGGCTCACAACTT-3', 1D_V259D_R: 5'-CATGTTTTGTGCATTTTTCCA-3', D1_1336_54_F: 5'-GGCCACTCATGGGTCTTCT-3' and D1_1336_54_R: 5'-AGATTGGGGCTACCCAGTG-3'.

Statistical analysis

Fisher's exact and Mann-Whitney U tests were used for statistical evaluation, with $p < 0.05$ considered to be significant. Results are expressed as mean $\pm$ SD (standard deviation).

Results

The pathological and clinical characteristics of the 97 patients with PH are presented in Table 1. This included 35 subjects from Yale University with previously published mutational status for whom histological slides were available for review,²² 32 additional patients from Yale University, 19 additional patients from Düsseldorf University and 11 additional samples from the University of Bonn.

On gross pathology, uninodular adrenocortical adenoma with or without surrounding hyperplasia was found in 83 cases (Fig. 1a, c), whereas 6 displayed multiple adrenocortical

nodules (Fig. 1b), and 7 demonstrated diffuse hyperplasia (Fig. 1d); 1 sample could not be assessed due to fragmentation (Table 1). Sanger sequencing revealed 36 *KCNJ5* mutations (37.1%). 32 of these were previously described (23 G151R, 9 L168R).¹⁰ Four previously undescribed variants were identified (Fig. 2a, Table 2) - I157K, F154C and 2 insertions (I150_G151insM and I144_E145insAI). Normal tissue was only available for the case with I150_G151insM mutation and confirmed a somatic mutation. 34/36 *KCNJ5* mutations were found in uninodular APAs, 2 in multinodular tumors. Sampling of four nodules, one area of nodular hyperplasia and normal adrenal tissue from APA81 demonstrated the presence of an L168R *KCNJ5* mutation exclusively in the largest nodule, whereas all other samples were wild type for this mutation (Fig. 2). Targeted sequencing of *CACNA1D*, *ATP1A1*, *ATP2B3* and *CTNNB1* exons with reported mutations in APAs,²²⁻²⁴ in combination with exome sequencing²² revealed additional mutations in 23 APAs. 10 samples (10.3%) had mutations in *CACNA1D*, 8 in *ATP1A1* (8.2%), 3 in *ATP2B3* (3.1%) and 2 in *CTNNB1* (2.1%) (Table 1). Among these were previously undescribed mutations in *ATP2B3* (V426G_V427Q_A428_L433del) and in *CTNNB1* (A39Efs*3). Collectively, we identified mutations in specimens from 59 patients (60.8%). Where available, surrounding normal adrenal tissue showed wild type sequence, consistent with these mutations occurring somatically. Remarkably, two *CACNA1D* mutations were detected in samples with adrenal hyperplasia by exome sequencing and Sanger sequencing, respectively (APA62 and APA119, Fig. 1d, Table 1, Table 3). In the case of APA62, although the right adrenal vein could not be cannulated with certainty in two procedures, preoperative CT scan showed thickening of the left adrenal gland, and there was no recurrence of hypertension upon follow-up, consistent with unilateral disease.

On CT, tumors harboring a *KCNJ5* mutation displayed a statistically greater diameter (1.25-fold larger) versus those with identified mutations in *CACNA1D*, *ATP1A1*, *ATP2B3*, or *CTNNB1* (19.1 ± 5.5 vs. 15.3 ± 4.4 mm; $p=0.023$). On average, *KCNJ5*-mutant tumors were 1.85-fold larger in area (mm^2) when compared to tumors with other identified mutations ($p=0.002$). Pre-contrast Hounsfield units (H.U.) were also significantly different between *KCNJ5*-mutant tumors and those with identified mutations in other genes (3.2 ± 10.6 vs. 20.4 ± 12.9 H.U., $p=0.0002$; Figure 3). By evaluating the 95% confidence intervals for all non-*KCNJ5* mutants, tumors greater than 17.4 mm in diameter and greater than 201.6 mm^2 in area are more likely to harbor a *KCNJ5* mutation. Likewise, tumors with average H.U. less than 14.0 have an increased likelihood of containing *KCNJ5* mutations. 53.3% of all tumors with *KCNJ5* mutations and available CT data had diameter greater than 17.4 mm and H.U. less than 14.0, compared with 5.6% of non-*KCNJ5* mutants (specificity of 94.4%, sensitivity of 53.3%).

34 of 36 tumors exhibiting *KCNJ5* mutations demonstrated the classic histological pattern of APAs (Figure 1), with >50% of the tumor comprising of large clear cells with abundant microvesicular cytoplasm with numerous lipid droplets ("fasciculata-like"²³). The histological pattern of tumors with *CACNA1D* and ATPase mutations was heterogeneous. 3/10 *CACNA1D*-positive tumors were "fasciculata-like", 7/10 were predominantly composed of polygonal cells with eosinophilic cytoplasm ("reticulosa-like") and small cells with high nuclear cytoplasmic ratio ("glomerulosa-like") (Figure 1E, F). 3/8 of the *ATP1A1* positive samples, 2/3 *ATP2B3*-positive tumors and 1/2 *CTNNB1*-positive tumors had >50%

clear cells. The fraction with “fasciculata-like” clear cells was not significantly different among tumors with mutations in *CACNA1D*/ATPase/*CTNNB1* (9/23) and tumors without identified mutations (22/38, $p=0.19$). *KCNJ5*-positive specimens differed significantly from both these groups ($p=5\times 10^{-6}$ vs. other mutations, $p=0.0003$ vs. wild type). Intracellular eosinophilic proteinaceous globules were similarly frequent in the group with *KCNJ5* mutations (4/36) and in those with non-*KCNJ5* mutations (5/23) ($p=0.46$)²³. The *CACNA1D*/ATPase/*CTNNB1* mutant tumors showed monomorphic bland nuclei in 22/23 cases, whereas variations in nuclear size and shape (nuclear pleomorphism) were present in 25/36 tumors with *KCNJ5* mutations and 17/38 tumors without any identified mutations ($p=4\times 10^{-7}$ versus *KCNJ5* and $p=0.001$ versus non-mutant). None of the tumors showed mitoses, necrosis or other features of malignancy.

The majority of patients with *KCNJ5* mutations were female (25/36) despite the unusual male preponderance in our cohort, whereas the majority of patients with mutations in other genes were male (16/23, $p=0.007$). In the remaining cohort, 12/38 patients were female ($p=0.002$ vs. *KCNJ5* and $p=1$ vs. other mutations).

In line with preoperative CT assessment, pathology showed that lesions with *KCNJ5* mutations were significantly larger (19.6 ± 6.5 mm) than those with non-*KCNJ5* mutations (14.5 ± 5.8 mm, $p=0.001$) or without mutations (17.7 ± 15.5 mm, $p=0.008$).

Discussion

These findings confirm previous observations on somatic mutations in *KCNJ5*, *CACNA1D*, *ATP1A1*, *ATP2B3* and *CTNNB1* in APAs. Interestingly, the fraction of tumors with mutations was considerably higher in samples from Bonn (90.9%) and Düsseldorf (80.0% of adenomas, 63.2% of all samples, including hyperplasias) than in samples from Yale (54.7% of adenomas, 55.2% of all samples), possibly due to sampling bias for Bonn cases (exclusion of tumors <1 cm), referral bias or differences in standards of case detection and AVS, an obvious limitation of our study that is due to its multicentric nature. Unfortunately, retrospective analysis of cases referred for surgery did not always allow for assessment of the original diagnostic biochemistry.

We identified a surprisingly high number of novel *KCNJ5* mutations, including two missense mutations and two in-frame insertions. The proximity of these variants to the selectivity filter and to the previously identified mutations suggests that they likely cause loss of potassium over sodium selectivity; however this remains to be proven by electrophysiology. Similarly, the novel indel variant in *ATP2B3* is located close to previously described deletions. While the novel *CTNNB1* variant is also located close to previously described mutations, it causes a frame shift, leading to early termination of the protein. Because somatic *CTNNB1* mutations are typically gain of function, the role of this A39Efs*3 mutation remains unclear.

The prevalence of *CACNA1D* mutations in our cohort may be an underestimate – only known mutational hotspots were screened. In 2 cases, *CACNA1D* mutations were undetectable by Sanger sequencing and only recognized by exome sequencing (10.1% of all

reads in APA62, 16.5% in APA68). Remarkably, we identified *CACNA1D* mutations in two samples with diffuse hyperplasia (APA62, APA119), extending the spectrum of lesions with such mutations beyond APAs. Similar findings were recently reported for *KCNJ5*³². To our knowledge, this also represents the first reported exome of an adrenal hyperplasia specimen with primary aldosteronism.

In aldosterone-producing adenomas, the correlation between the imaging phenotype, histology and genotype is intriguing. The high fat content in lesions with a fasciculata-like appearance (Fig. 1) explains lower Hounsfield units on CT scans (Fig. 3), and the presence of this histological feature in almost all *KCNJ5*-positive tumors studied here explains the association with lower pre-contrast Hounsfield units on CT scans. The high prevalence of fasciculata-like APAs with *KCNJ5* mutations explains the overall predominance of this histopathological phenotype. The histological features of zona fasciculata do not appear to be associated with cortisol secretion in the vast majority of cases, although rare cases of adenomas secreting both cortisol and aldosterone with *KCNJ5* mutations have been described.^{33, 34} *KCNJ5*-positive tumors also appear to be larger than others. This may have a direct clinical implication. It has previously been suggested that adrenal venous sampling may not be necessary in patients younger than age 40 with solitary unilateral apparent adenoma²; however, the cut-off for age is debated. In line with this suggestion, we propose that young, especially female, patients with larger tumors displaying lower H.U. on CT scan, suggesting the presence of a *KCNJ5* mutant tumor, may be able to avoid preoperative adrenal vein sampling.

Of note, previous studies have found conflicting results regarding the histological phenotype of mutations in APAs, which has been attributed to sample heterogeneity.^{12, 15, 23, 35}

We also identify a multinodular gland with *KCNJ5* mutation in only one of the nodules, similar to data reported by Azizan et al. for a tumor with *ATP1A1* mutation.²³ The majority of tumors with *KCNJ5* mutations are uninodular, and the molecular basis of proliferation in mutation-negative nodules of multinodular glands remains unclear. Possibly, genetic or epigenetic factors distinct from *KCNJ5* mutations trigger proliferation in these cases. The occurrence of a *KCNJ5* mutation in one of the proliferating cells may lead to proliferative advantage (largest nodule) and aldosterone secretion. Unlike a previous publication,²³ we did not find a clear association of mutations in *ATP1A1* and *CACNA1D* with a “glomerulosa-like” histopathological profile. Specimens with ATPase, *CACNA1D* and *CTNNB1* mutations were histologically heterogeneous, similar to those without mutations, with the exception of a conspicuous absence of nuclear atypia. In summary, *KCNJ5* mutations appear to be associated with a distinct imaging and histopathology phenotype. The molecular basis of these features, however, remains unexplained.

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References

1. Rossi GP, Bernini G, Caliumi C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *Journal of the American College of Cardiology*. 2006; 48:2293–2300. [PubMed: 17161262]
2. Funder JW, Carey RM, Fardella C, et al. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism*. 2008; 93:3266–3281. [PubMed: 18552288]
3. Mosso L, Carvajal C, Gonzalez A, et al. Primary aldosteronism and hypertensive disease. *Hypertension*. 2003; 42:161–165. [PubMed: 12796282]
4. Milliez P, Girerd X, Plouin PF, et al. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *Journal of the American College of Cardiology*. 2005; 45:1243–1248. [PubMed: 15837256]
5. Young WF. Primary aldosteronism: renaissance of a syndrome. *Clinical Endocrinology*. 2007; 66:607–618. [PubMed: 17492946]
6. Sawka AM, Young WF, Thompson GB, et al. Primary aldosteronism: factors associated with normalization of blood pressure after surgery. *Annals of Internal Medicine*. 2001; 135:258–261. [PubMed: 11511140]
7. Zarnegar R, Young WF Jr, Lee J, et al. The aldosteronoma resolution score: predicting complete resolution of hypertension after adrenalectomy for aldosteronoma. *Annals of Surgery*. 2008; 247:511–518. [PubMed: 18376197]
8. Walz MK, Gwosdz R, Levin SL, et al. Retroperitoneoscopic adrenalectomy in Conn's syndrome caused by adrenal adenomas or nodular hyperplasia. *World Journal of Surgery*. 2008; 32:847–853. [PubMed: 18343972]
9. Steichen O, Zinzindohoue F, Plouin PF, et al. Outcomes of adrenalectomy in patients with unilateral primary aldosteronism: a review. *Hormone and Metabolic Research*. 2012; 44:221–227. [PubMed: 22395801]
10. Choi M, Scholl UI, Yue P, et al. K⁺ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science*. 2011; 331:768–772. [PubMed: 21311022]
11. Akerstrom T, Crona J, Delgado Verdugo A, et al. Comprehensive Re-Sequencing of Adrenal Aldosterone Producing Lesions Reveal Three Somatic Mutations near the KCNJ5 Potassium Channel Selectivity Filter. *PLoS ONE*. 2012; 7:e41926. [PubMed: 22848660]
12. Boulkroun S, Beuschlein F, Rossi GP, et al. Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism. *Hypertension*. 2012; 59:592–598. [PubMed: 22275527]
13. Scholl UI, Lifton RP. New insights into aldosterone-producing adenomas and hereditary aldosteronism: mutations in the K⁺ channel KCNJ5. *Current Opinion in Nephrology and Hypertension*. 2013; 22:141–147. [PubMed: 23318698]
14. Zheng FF, Zhu LM, Nie AF, et al. Clinical characteristics of somatic mutations in Chinese patients with aldosterone-producing adenoma. *Hypertension*. 2015; 65:622–628. [PubMed: 25624344]
15. Fernandes-Rosa FL, Williams TA, Riester A, et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension*. 2014; 64:354–361. [PubMed: 24866132]
16. Taguchi R, Yamada M, Nakajima Y, et al. Expression and mutations of KCNJ5 mRNA in Japanese patients with aldosterone-producing adenomas. *Journal of Clinical Endocrinology and Metabolism*. 2012; 97:1311–1319. [PubMed: 22278422]
17. Wang B, Li X, Zhang X, et al. Prevalence and characterization of somatic mutations in chinese aldosterone-producing adenoma patients. *Medicine*. 2015; 94:e708. [PubMed: 25906099]
18. Wu VC, Huang KH, Peng KY, et al. Prevalence and clinical correlates of somatic mutation in aldosterone producing adenoma-Taiwanese population. *Sci Rep*. 2015; 5:11396. [PubMed: 26066391]
19. Lenzini L, Rossitto G, Maiolino G, et al. A Meta-Analysis of Somatic KCNJ5 K Channel Mutations In 1636 Patients With an Aldosterone-Producing Adenoma. *Journal of Clinical Endocrinology and Metabolism*. 2015:jc20152149.

20. Spat A, Hunyady L. Control of aldosterone secretion: a model for convergence in cellular signaling pathways. *Physiological Reviews*. 2004; 84:489–539. [PubMed: 15044681]
21. Oki K, Plonczynski MW, Luis Lam M, et al. Potassium channel mutant KCNJ5 T158A expression in HAC-15 cells increases aldosterone synthesis. *Endocrinology*. 2012; 153:1774–1782. [PubMed: 22315453]
22. Scholl UI, Goh G, Stolting G, et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nature Genetics*. 2013; 45:1050–1054. [PubMed: 23913001]
23. Azizan EA, Poulsen H, Tuluc P, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nature Genetics*. 2013; 45:1055–1060. [PubMed: 23913004]
24. Beuschlein F, Boulkroun S, Osswald A, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nature Genetics*. 2013; 45:440–444. [PubMed: 23416519]
25. Williams TA, Monticone S, Schack VR, et al. Somatic ATP1A1, ATP2B3, and KCNJ5 mutations in aldosterone-producing adenomas. *Hypertension*. 2014; 63:188–195. [PubMed: 24082052]
26. Tadjine M, Lampron A, Ouadi L, et al. Frequent mutations of beta-catenin gene in sporadic secreting adrenocortical adenomas. *Clinical Endocrinology*. 2008; 68:264–270. [PubMed: 17854394]
27. Berthon A, Sahut-Barnola I, Lambert-Langlais S, et al. Constitutive beta-catenin activation induces adrenal hyperplasia and promotes adrenal cancer development. *Human Molecular Genetics*. 2010; 19:1561–1576. [PubMed: 20106872]
28. Young WF Jr. Clinical practice. The incidentally discovered adrenal mass. *N Engl J Med*. 2007; 356:601–610. [PubMed: 17287480]
29. Harvey A, Kline G, Pasieka JL. Adrenal venous sampling in primary hyperaldosteronism: comparison of radiographic with biochemical success and the clinical decision-making with “less than ideal” testing. *Surgery*. 2006; 140:847–853. discussion 853–845. [PubMed: 17188130]
30. Vonend O, Ockenfels N, Gao X, et al. Adrenal venous sampling: evaluation of the German Conn's registry. *Hypertension*. 2011; 57:990–995. [PubMed: 21383311]
31. Vincent JM, Morrison ID, Armstrong P, et al. The size of normal adrenal glands on computed tomography. *Clinical Radiology*. 1994; 49:453–455. [PubMed: 8088036]
32. Zhang J, Li N, Hu Y, et al. [Sequence analysis of coding regions of KCNJ5 gene in unilateral adrenal hyperplasia]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi (Chinese Journal of Medical Genetics)*. 2015; 32:21–25. [PubMed: 25636093]
33. Yamada M, Nakajima Y, Taguchi R, et al. KCNJ5 mutations in aldosterone- and cortisol-co-secreting adrenal adenomas [Rapid Communication]. *Endocrine Journal*. 2012; 59:735–741. [PubMed: 22863749]
34. Thiel A, Reis AC, Haase M, 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. [PubMed: 25750087]
35. Azizan EA, Lam BY, Newhouse SJ, et al. Microarray, qPCR, and KCNJ5 sequencing of aldosterone-producing adenomas reveal differences in genotype and phenotype between zona glomerulosa- and zona fasciculata-like tumors. *Journal of Clinical Endocrinology and Metabolism*. 2012; 97:E819–829. [PubMed: 22442279]

Figure 1. Gross pathology and hematoxylin-eosin staining of representative cases of uninodular and multinodular aldosterone producing adrenal lesions

A) APA55. Solitary uninodular adrenocortical 1.9 cm adenoma (arrow) with unremarkable cortex and surrounding fat tissue; tumor has the *KCNJ5*-G151R mutation. B) APA60. Macronodular adrenocortical hyperplasia with at least 3 distinct macronodules (arrows); no mutations were identified. C) APA50. Uninodular tumor with surrounding adrenocortical hyperplasia; tumor has an *ATP2B3* V426_V427del mutation. D) APA62. Diffuse adrenocortical hyperplasia. Specimen carries the *CACNA1D* F767V mutation. E) APA57. H&E staining of APA displays cells with large clear microvesicular cytoplasm with

numerous lipid droplets; tumor is *KCNJ5* G151R positive (x40), F) APA59. H&E staining of APA showing cells with compact lipid sparse eosinophilic cytoplasm arranged in trabeculae. Occasional cells in the center show intracytoplasmic brown pigment consistent with lipofuscin; tumor is *CACNA1D* G403R positive. Scale bars: 100 μ m.

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samples are wild type for this mutation. Corresponding Sanger sequences are depicted below.

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Figure 3. Representative CT scans demonstrating findings typical of different genotypes of aldosterone-producing adenomas

A) *KCNJ5*-mutant left aldosteronoma displaying larger tumor size and lower average H.U. reading. Left aldosterone-producing tumors with *CACNA1D* (B) and *ATP1A1* (C) mutations, respectively, demonstrating smaller tumor size and higher H.U. D) Wild type left aldosteronoma.

Table 1

Characteristics of patients with unilateral primary hyperaldosteronism

Tumor	Origin	Age	Sex	ARR	Serum K+ (mmol/L)	Exome	KCNJ5	CACNA1D	ATP1A1	ATP2B3	CTNNB1	Side	Size (cm)	1 >1 nodule	Max Diameter (mm, CT)	Area (mm ² , CT)	HU pre-contrast	% clear cells	Non-tumor cortex	Nuclear atypia
APA105	Düsseldorf	44	M	>783**	3.8	no	F154C/+	WT	WT	WT	WT	right	4.0	1	NA	NA	NA	middle	NA	2+
APA71	Yale	60	M	367	3.1	no	G151R/+	N/A	N/A	N/A	N/A	left	2.8	1	29	783	3	high	normal	0
APA72	Yale	40	F	165	3.9	no	G151R/+	WT	WT	WT	N/A	right	1.5	1	19	247	-7	high	normal	3+
APA73	Bonn	40	F	174*	3.4	no	G151R/+	WT	WT	WT	WT	left	1.7	1	NA	NA	NA	high	normal	0
APA78	Bonn	43	F	358	3.0	no	G151R/+	WT	WT	WT	WT	left	2.7	1	NA	NA	NA	high	hyp	0
APA100	Düsseldorf	52	F	>646**	3.0	no	G151R/+	WT	WT	WT	WT	left	1.5	1	18.5	204	-4	low	hyp	0
APA25	Yale	45	M	100	3.1	yes	G151R/+	WT	WT	WT	WT	left	1.5	1	21	315	-7	high	hyp	2+
APA57	Yale	34	M	elevated	2.6	no	G151R/+	WT	WT	WT	WT	left	2.1	1	26	572	-2	middle*	normal	1+
APA63	Yale	43	M	elevated	3.2	no	G151R/+	WT	WT	WT	WT	left	1.3	1	15	150	20	middle*	normal	3+
APA118	Yale	45	F	>100	2.2	no	G151R/+	WT	WT	WT	WT	left	1.3	1	20	340	NA	high	hyp	0
APA74	Bonn	73	F	15*	3.3	no	G151R/+	WT	WT	WT	WT	right	2.2	1	NA	NA	NA	high	normal	1+
APA75	Bonn	53	F	63*	3.1	no	G151R/+	WT	WT	WT	WT	right	2.5	1	NA	NA	NA	high	normal	1+
APA76	Bonn	44	F	N/A	2.9	no	G151R/+	WT	WT	WT	WT	right	2.5	1	NA	NA	NA	high	normal	1+
APA77	Bonn	35	M	568*	2.9	no	G151R/+	WT	WT	WT	WT	right	2.5	1	NA	NA	NA	high	normal	2+
APA94	Düsseldorf	64	F	93**	2.6	no	G151R/+	WT	WT	WT	WT	right	2.0	1	NA	NA	NA	high	NA	1+
APA97	Düsseldorf	46	F	>169**	4.2	no	G151R/+	WT	WT	WT	WT	right	2.2	1	19	168	-14	high	hyp	0
APA106	Düsseldorf	60	F	56**	3.3	no	G151R/+	WT	WT	WT	WT	right	1.2	1	10	100	7	high	normal	0
APA28	Yale	52	F	elevated	2.9	yes	G151R/+	WT	WT	WT	WT	right	1.6	1	13	156	26	middle*	normal	1+
APA44	Yale	44	M	52	3.4	no	G151R/+	WT	WT	WT	WT	right	1.1	1	NA	NA	NA	high	normal	1+
APA55	Yale	40	M	110	1.4	no	G151R/+	WT	WT	WT	WT	right	1.9	1	NA	NA	NA	high	normal	1+
APA56	Yale	59	F	126	2.8	no	G151R/+	WT	WT	WT	WT	right	1.8	1	17	204	11	high	normal	3+
APA117	Yale	41	F	280	3.8	no	G151R/+	WT	WT	WT	WT	right	1.5	1	16	176	7	high	normal	3+
APA123	Yale	34	M	23	1.9	no	G151R/+	WT	WT	WT	WT	right	1.7	1	18	270	NA	high	normal	1+
APA125	Yale	54	M	71	3.6	no	G151R/+	WT	WT	WT	WT	right	1.5	1	19	209	NA	high	hyp	0
APA79	Bonn	51	M	251*	2.9	no	1150_G151 insM/+	WT	WT	WT	WT	right	1.4	1	NA	NA	NA	high	normal	0
APA121	Yale	52	F	239	3.2	no	1157K/+	WT	WT	WT	WT	left	1.5	1	NA	NA	NA	high	normal	2+
APA80	Yale	42	F	N/A	4.7	no	L168R/+	WT	N/A	WT	WT	left	3.2	>1	NA	NA	NA	middle*	normal	1+

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Tumor	Origin	Age	Sex	ARR	Serum K ⁺ (mmol/ L)	Exome	KCNJ5	CACNA1D	ATPIA1	ATP2B3	CTNNB1	Side	Size (cm)	1 / >1 nodule	Max Diameter (mm, CT)	Area (mm ² , CT)	HU pre- contrast	% clear cells	Non-tumor cortex	Nuclear atrophia
APA81	Bonn	48	F	elevated	3.0	no	L168R/+	WT	WT	WT	WT	left	1.4	>1	NA	NA	NA	high	hyp	1+
APA103	Düsseldorf	55	F	46**	3.0	no	L168R/+	WT	WT	WT	WT	left	2.0	1	22	380	5	high	NA	3+
APA46	Yale	48	F	elevated	2.9	no	L168R/+	WT	WT	WT	WT	left	1.2	1	NA	NA	NA	high	normal	0
APA52	Yale	37	F	79	3.4	no	L168R/+	WT	WT	WT	WT	left	2.3	1	NA	NA	NA	high	normal	3+
APA82	Bonn	42	F	>23.1*	3.1	no	L168R/+	WT	WT	WT	WT	right	1.5	1	NA	NA	NA	high	normal	0
APA47	Yale	59	F	295	2.6	no	L168R/+	WT	WT	WT	WT	right	2.8	1	NA	NA	NA	high	normal	3+
APA122	Yale	37	F	650	3.4	no	L168R/+	WT	WT	WT	WT	right	1.6	1	15	210	6	high	normal	2+
APA127	Yale	28	F	67	3.2	no	L168R/+	WT	WT	WT	WT	right	2.0	1	14	140	3	high	normal	3+
APA102	Düsseldorf	32	F	178**	2.2	no	S143_I144 insIA/+	WT	WT	WT	WT	left	2.9	1	32	704	−6	high	NA	2+
APA62	Yale	46	M	43	3.4	yes	WT	F767V/+	WT	WT	WT	left	hyp	hyp	NA	NA	NA	low	hyp	0
APA68	Yale	55	M	N/A	3.0	yes	WT	F767V/+	WT	WT	WT	right	1.5	1	13	130	6	middle*	normal	0
APA59	Yale	57	M	74	3.3	no	WT	G403R/+ (8A)	WT	WT	WT	left	1.6	1	13	117	39	low	normal	0
APA65	Yale	59	M	128	3.8	no	WT	G403R/+ (8A)	WT	WT	WT	left	2.0	1	22	352	36	middle	hyp	0
APA113	Yale	55	M	>70	3.4	no	WT	G403R/+ (8A)	WT	WT	WT	left	1.4	1	12	108	30	low	normal	0
APA31	Yale	64	M	120	3.3	yes	WT	G403R/+ (8A)	WT	WT	WT	right	1.0	1	10	90	17	low	normal	0
APA83	Yale	47	M	90	3.1	no	WT	G403R/+ (8B)	WT	WT	WT	right	0.8	1	12	120	8	high	hyp	0
APA119	Yale	44	M	elevated	3.4	no	WT	Gly403R/+ (8B)	WT	WT	WT	left	1.3	hyp	13	117	3	low	hyp	0
APA29	Yale	42	F	340	3.0	yes	WT	I770M/+	WT	WT	WT	right	1.3	1	12	132	11	high	normal	0
APA109	Düsseldorf	39	F	300**	4.2	no	WT	V1338M/+	WT	WT	WT	right	1.0	1	18	108	34	low	normal	0
APA84	Bonn	47	M	N/A	3.0	no	WT	WT	F100_L104 del/+	WT	WT	right	1.5	1	NA	NA	NA	low	normal	0
APA89	Yale	34	M	20	4.0	no	WT	WT	L104R/+	N/A	WT	right	2.2	1	19	266	15	middle*	hyp	0
APA107	Düsseldorf	69	M	>571**	3.5	no	WT	WT	L104R/+	WT	WT	left	2.5	1	25	308	6	middle	NA	0
APA85	Yale	63	M	32	3.8	no	WT	WT	L104R/+	WT	WT	left	1.1	1	11	99	10	high	normal	0
APA98	Düsseldorf	54	F	81**	4.2	no	WT	WT	L104R/+	WT	WT	right	1.3	1	10	80	22	low	normal	0
APA104	Düsseldorf	64	M	101**	3.0	no	WT	WT	L104R/+	WT	WT	right	0.8	1	11	72	44	low	normal	0
APA112	Düsseldorf	71	F	60**	3.5	no	WT	WT	L104R/+	WT	WT	right	0.9	1	18	91	17	high	normal	0
APA86	Yale	52	M	317	4.0	no	WT	WT	L104R/+	WT	WT	right	1.5	1	17	170	33	low	hyp	0
APA49	Yale	59	F	26	2.4	no	WT	WT	WT	L425_V426del/+	WT	left	0.9	1	NA	NA	NA	middle*	hyp	0
APA50	Yale	35	M	160	2.4	no	WT	WT	WT	V426_V427del	WT	left	1.2	1	15	180	11	middle*	hyp	0
APA124	Yale	33	M	138	4.1	no	WT	WT	WT	V426G_V427Q_A428_I433del	WT	left	1.0	1	18	182	NA	low	hyp	0

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Tumor	Origin	Age	Sex	ARR	Serum K ⁺ (mmol/L)	Exome	KCNJ5	CACNA1D	ATPIA1	ATP2B3	CTNNB1	Side	Size (cm)	1 / >1 node	Max Diameter (mm, CT)	Area (mm ² , CT)	HU pre-contrast	% clear cells	Non-tumor cortex	Nuclear atypia
APA116	Yale	24	F	21	2.9	no	WT	WT	WT	WT	A39Efs*3/+	left	2.0	1	21	315	25	low	normal	2+
APA53	Yale	65	F	elevated	3.9	no	WT	WT	WT	WT	S45P/+	left	3.0	1	NA	NA	NA	middle*	normal	0
APA93	Bonn	68	M	37	2.9	no	WT	WT	WT	WT	WT	left	2.5	1	NA	NA	NA	middle*	hyp	0
APA95	Düsseldorf	55	M	>212**	3.6	no	WT	WT	WT	WT	WT	left	hyp	hyp	20	160	4	low	hyp	0
APA99	Düsseldorf	62	F	elevated	3.4	no	WT	WT	WT	WT	WT	left	frag	frag	19	132	6	middle	hyp	0
APA24	Yale	40	M	85	2.5	yes	WT	WT	WT	WT	WT	left	3.0	1	NA	NA	NA	high	normal	3+
APA26	Yale	48	M	30	5.0	yes	WT	WT	WT	WT	WT	left	0.6	1	13	156	23	low	hyp	0
APA27	Yale	35	F	7	4.1	yes	WT	WT	WT	WT	WT	left	1.0	1	17	221	8	high	normal	0
APA30	Yale	50	M	1640	2.7	yes	WT	WT	WT	WT	WT	left	2.0	1	25	425	9	high	hyp	3+
APA39	Yale	38	F	177	3.1	no	WT	WT	WT	WT	WT	left	2.5	1	NA	NA	NA	high	normal	3+
APA40	Yale	35	F	87	4.2	no	WT	WT	WT	WT	WT	left	hyp	hyp	NA	NA	NA	middle*	hyp	0
APA42	Yale	68	M	elevated	3.1	no	WT	WT	WT	WT	WT	left	2.0	1	NA	NA	NA	low	hyp	0
APA43	Yale	44	M	40	3.5	no	WT	WT	WT	WT	WT	left	0.7	>1	NA	NA	NA	low	hyp	0
APA45	Yale	44	F	elevated	3.4	no	WT	WT	WT	WT	WT	left	1.2	1	NA	NA	NA	middle*	normal	0
APA48	Yale	61	M	74	3.0	no	WT	WT	WT	WT	WT	left	1.6	1	17	153	25	middle	hyp	3+
APA51	Yale	64	M	43	4.2	yes	WT	WT	WT	WT	WT	left	1.1	1	NA	NA	NA	high	hyp	3+
APA58	Yale	36	F	47	3.2	no	WT	WT	WT	WT	WT	left	1.0	1	NA	NA	NA	high	hyp	2+
APA60	Yale	37	F	elevated	2.3	no	WT	WT	WT	WT	WT	left	0.8	>1	9	63	4	middle*	hyp	0
APA61	Yale	43	M	52	2.9	no	WT	WT	WT	WT	WT	left	1.0	1	NA	NA	NA	high	hyp	1+
APA67	Yale	45	F	elevated	3.4	yes	WT	WT	WT	WT	WT	left	1.8	1	19	285	23	low	normal	2+
APA69	Yale	72	M	N/A	4.0	yes	WT	WT	WT	WT	WT	left	0.5	1	NA	NA	NA	low	normal	0
APA70	Yale	54	M	70	3.4	no	WT	WT	WT	WT	WT	left	1.4	1	14	140	27	low	normal	0
APA87	Yale	41	M	355	3.0	no	WT	NA	N/A	N/A	WT	left	1.5	1	NA	NA	NA	high	normal	3+
APA88	Yale	47	F	76	3.9	no	WT	NA	N/A	N/A	WT	right	2.2	1	NA	NA	NA	middle*	normal	3+
APA90	Yale	64	M	133	3.2	no	WT	WT	WT	WT	WT	left	1.2	1	17	272	30	middle	hyp	1+
APA91	Yale	58	M	80	3.2	no	WT	WT	WT	WT	WT	left	0.8	1	9	54	27	low	normal	0
APA92	Yale	44	M	410	2.7	no	WT	WT	WT	WT	WT	left	1.1	>1	12	120	29	middle*	hyp	0
APA120	Yale	34	M	350	2.7	no	WT	WT	WT	WT	WT	left	2.0	1	23	345	4	high	hyp	1+
APA96	Düsseldorf	60	M	85**	3.8	no	WT	WT	WT	WT	WT	right	N/A	1	25	275	4	high	normal	0
APA101	Düsseldorf	51	M	120**	2.8	no	WT	WT	WT	WT	WT	right	hyp	hyp	21	210	27	middle	NA	0

Tumor	Origin	Age	Sex	ARR	Serum K ⁺ (mmol/L)	Exome	KCNJ5	CACNA1D	ATP1A1	ATP2B3	CTNNB1	Side	Size (cm)	1 / >1 nodule	Max Diameter (mm, CT)	Area (mm ² , CT)	HU pre-contrast	% clear cells	Non-tumor cortex	Nuclear atypia
APA108	Düsseldorf	52	M	Elevated	4.2	no	WT	WT	WT	WT	WT	right	0.9	1	32	544	NA	high	hyp	0
APA110	Düsseldorf	65	M	>87**	4.1	no	WT	WT	WT	WT	WT	right	hyp	hyp	8	48	6	middle	normal	0
APA111	Düsseldorf	46	M	124**	3.5	no	WT	WT	WT	WT	WT	right	hyp	hyp	NA	NA	NA	middle	hyp	0
APA41	Yale	43	M	elevated	3.6	no	WT	WT	WT	WT	WT	right	2.1	>1	NA	NA	NA	high	normal	1+
APA54	Yale	69	M	70	3.0	yes	WT	WT	WT	WT	WT	right	1.7	1	NA	NA	NA	low	hyp	0
APA64	Yale	74	M	elevated	3.5	yes	WT	WT	WT	WT	WT	right	1.1	1	9	45	NA	middle*	hyp	1+
APA66	Yale	54	F	elevated	4.4	yes	WT	WT	WT	WT	WT	right	0.6	1	60	2700	31	middle*	normal	1+
APA114	Yale	55	F	NA	4.2	no	WT	WT	WT	WT	WT	right	6.0	1	60	2220	33	middle	normal	1+
APA115	Yale	48	M	90	3	no	WT	WT	WT	WT	WT	right	8.0	1	8	48	6	high	hyp	0
APA126	Yale	36	F	94	3.2	no	WT	WT	WT	WT	WT	right	1.1	1	NA	NA	NA	high	hyp	2+

Reference ranges: Serum potassium (K⁺) 3.5-5.0 mmol/l; ARR, > 20 or >50 (*) indicative of primary hyperaldosteronism. **, aldosterone/PRC ratio, >50 indicative of primary hyperaldosteronism, 2, 28. F, female; M, male; N/A, not applicable or did not amplify; hyp, hyperplasia; frag, fragmented; Max, maximum; HU, Hounsfield Units; % clear cells: high=>66%, middle=33-66%, *>50%, low=<33%. See *Material and Methods* for details.

Table 2

Previously undescribed mutations in aldosterone-producing adenomas

Chr	Position	Base change	Gene	Effect on cDNA	Effect on protein
11	128,781,618_128,781,619	insATG	<i>KCNJ5</i>	c.450_451insATG	I150_G151insM
11	128,781,629	T>G	<i>KCNJ5</i>	c.461T>G	F154C
11	128,781,638_128,781,639	TC>AA	<i>KCNJ5</i>	c.470_471delinsAA	I157K
11	128,781,601_128,781,602	insCCATTG	<i>KCNJ5</i>	c.433_434insCCATTG	I144_E145insAI
3	41,266,119_41,266,141	delCCACTACCACAGCTCCTTCTCTG	<i>CTNNB1</i>	c.116_138del	A39Efs*3
X	152,814,250_152,814,272	insGACA_delTCGTGGCTGTCCACAGAGGCCT	<i>ATP2B3</i>	c.1276_1298 insGACA_delTCGTGGCTGTCCACAGAGGCCT	V426G_V427Q_A428_L433del

Chr, chromosome; position in Human genome build 19

Table 3
Candidate somatic mutations in APA62 with diffuse hyperplasia detected by exome sequencing

Chr	Position	Base change	Gene	Effect on protein	# of reads from tumor			# of reads from blood		p-value
					Ref. allele	Non-ref. allele	% of all reads	Ref. allele	Non-ref. allele	
3	53764486	T>G	<i>CACNA1D</i>	F767V	232	26	10.08	163	0	2.74×10^{-6}
15	51991560	G>A	<i>SCG3</i>	E344K	655	72	9.90	342	1	1.35×10^{-11}
3	120876376	A>G	<i>STXBPL5L</i>	H260R	353	46	11.53	222	1	1.31×10^{-8}

Chr, chromosome; position in Human genome build 19; ref, reference; p-value, significance of difference in frequency of non-reference allele between tumor and blood sequence. Only protein-coding somatic mutations are shown. *CACNA1D*, calcium channel, voltage-dependent, L type, alphaD; *SCG3*, secretogranin III; *STXBPL5L*, syntaxin binding protein 5-like