Primary Hyperaldosteronism

Somatic Mutations Affecting the Selectivity Filter of KCNJ5 Are Frequent in 2 Large Unselected Collections of Adrenal Aldosteronomas

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Abstract—Primary hyperaldosteronism, one cause of which is aldosterone-producing adenomas (APAs), may account for ≤5% to 10% of cases of essential hypertension. Germline mutations have been identified in 2 rare familial forms of primary hyperaldosteronism, but it has been reported recently that somatic mutations of the KCNJ5 gene, which encodes a potassium channel, are present in some sporadic nonsyndromic APAs. To address this further we screened 2 large collections of sporadic APAs from the United Kingdom and Australia (totalling 73) and found somatic mutations in the selectivity filter of KCNJ5 in 41% (95% CI: 31% to 53%) of the APAs (30 of 73). These included the previously noted nonsynonymous mutations, G151R and L158R, and an unreported 3-base deletion, delI157, in the region of the selectivity filter. APAs containing a somatic KCNJ5 mutation were significantly larger than those without (1.61 cm [95% CI: 1.39–1.83 cm] versus 1.04 cm [95% CI: 0.91–1.17 cm]; P<0.0001) but with substantial overlap in size between genotypes. The APAs carrying a mutation, but not those without, also consistently lacked a postural aldosterone response, suggesting a physiologically distinct subtype. Hence, somatic KCNJ5 mutations are not restricted to large APAs (>2 cm), and their frequency in our unselected series suggests they are common and could be important in the molecular pathogenesis of many sporadic cases of APA.

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Key Words: hyperaldosteronism ■ hypertension ■ potassium channels ■ KCNJ5 ■ aldosterone-producing adenoma ■ posture response

Primary hyperaldosteronism (PA) is now recognized as a common, treatable, and potentially curable form of hypertension, which may account for ≤10% of cases of so-called essential hypertension.1–3 Most cases of PA are sporadic and result from 2 major types of adrenal pathology, an aldosterone-producing adenoma (APA) or bilateral adrenal hyperplasia. In recently published series, the frequency of APA varied between 28% and 50% of patients with PA.4

Choi et al5 recently reported somatic mutations in a potassium channel, KCNJ5 (also called GIRK4 or Kir3.4), in 8 of 20 APAs studied and a germline mutation in the same gene in all 3 affected members of a family with florid, early onset, nondexamethasone-suppressible PA associated with marked hyperplasia of zona fasciculata (ZF), suggesting a novel pathway that might activate growth of aldosterone-secreting cells. These mutations within the selectivity filter of the potassium channel reduce the normal K⁺/Na⁺ selectivity of the channel, and the resulting depolarization of the adrenocortical cell could lead to calcium loading and growth. However, the APAs carrying KCNJ5 mutations were large (mean of 2.8 cm and all ≥2 cm in diameter) and might represent a subgroup with a phenotype more relevant to the giant hyperplastic adrenals seen in the family with the germline KCNJ5 mutation.5 To address this issue we have screened a large collection of APAs (totalling 73) from geographically distinct centers (United Kingdom and Australia) to determine whether somatic mutations of KCNJ5 are present in unselected APAs regardless of size. We also

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compared various phenotypic characteristics of mutation-bearing versus nonmutation-bearing APAs in an attempt to better understand the role of these mutations in APA development and phenotypic expression. Finally, we sought evidence of germline mutations in affected members of other families with nondexamethasone-suppressible PA attributed either to APA or bilateral adrenal hyperplasia (familial hyperaldosteronism type II [FH-II]).

Methods

Sample Collection

The APAs consisted of consecutive surgical adrenal specimens recovered at Addenbrooke’s Hospital United Kingdom (46 specimens) or at Greenslopes and Princess Alexandra Hospitals in Brisbane, Australia (27 specimens). Both are specialist referral centers for endocrine hypertension. For the United Kingdom tissues, nonadenoma adrenal tissue was also recovered, and at both centers an EDTA sample of peripheral blood was collected from each patient. All of the subjects taking part gave informed consent, and the study was approved by the local ethical review committee at each center. The patient characteristics are included in the Table. Eight FH-II pedigrees included affected members of 3 kindreds reported previously as demonstrating linkage at chromosome 7p22, as well as 5 smaller kindreds with the FH-II phenotype that are too small to establish linkage to the chromosome 7p22 locus.

Genomic DNA Extraction and Sequencing

Genomic DNA was extracted by standard methods. The genomic DNA from the APAs was recovered for somatic mutation detection, as well as genomic DNA from peripheral venous blood or adjacent nonadenoma adrenal tissue to confirm that any mutations detected in the APA were not present in the germline. The entire coding sequence (exons 2–3) and flanking regions of KCNJ5 were PCR amplified and the products purified with a 1-column clean-up method (Promega, Southampton, United Kingdom). Sanger sequencing of the purified products was undertaken on either the Applied Biosystems 3730xl DNA Analyzer or Beckman CEQ8000 platform. Please see the online-only Data Supplement for the PCR primer sequences.

Statistical Analysis

All of the data are presented as mean±SEM unless stated otherwise. Comparisons between groups were by ANOVA with post hoc testing or nonparametric ranking tests as appropriate using GraphPad Prism 5 for Windows software. Statistical significance was set at P<0.05.

Results

Sequencing of KCNJ5

The demographics of the 2 collections of APA were well matched (Table). Mutations were detected in 30 of 73 APAs (41% [95% CI: 31% to 53%]) and 20 of 46 APAs (44% [95% CI: 30% to 58%]) from the United Kingdom collection and 10 of 27 (38% [95% CI: 21% to 56%]) from the Australian collection. In all of the cases, the mutation could not be detected in genomic DNA from peripheral blood or normal adjacent adrenal tissue (United Kingdom samples only). An overall frequency of 41% is very similar to the smaller series reported by Choi et al.5 Both of the previously reported nonsynonymous somatic mutations in KCNJ5 were identified (G151R: n=11 in United Kingdom; n=8 in Australia; L168R: n=8 in United Kingdom; n=2 in Australia), as well as unreported deletion (delI157) in 1 of the United Kingdom APA (representative sequencing chromatograms are shown in Figure 1). No further new mutations or single nucleotide polymorphisms were detected in the exons or flanking regions of KCNJ5 in our collection. No somatic instances of T158A, the mendelian mutation reported in a family with florid PA and giant hyperplastic adrenals,5 were found.

Sequencing of FH-II Pedigrees

Sequencing of KCNJ5 in germline DNA from our FH-II pedigrees failed to identify any germline mutations. This was true whether the pedigree had been linked previously to the chromosome 7p22 locus.

Phenotypic Comparison of Patients With/Without Somatic KCNJ5 Mutations

The APAs carrying a KCNJ5 mutation were >50% larger, at 1.61 cm (95% CI: 1.39 to 1.83 cm), than those without a mutation, at 1.04 cm (95% CI: 0.91 to 1.17; P<0.0001), although the genotypes were indistinguishable (Figure 2) and overlap was obvious in both centers (please see Figure S1 in the online-only Data Supplement). Hence, APAs carrying a somatic mutation could not be reliably inferred from their size. We explored other genotype-phenotype associations in this data set and found a significant association of genotype with the aldosterone response to upright posture (calculated as the percentage rise in plasma aldosterone from 7:00 AM to 8:00 AM after overnight recumbency to 10:00 AM after 2 to 3 hours of standing, sitting, or walking, with “responsiveness” defined as a rise of ≥50% over basal).9,10 We only had these data for the Australian APAs, but in this collection 10 of 10 with the mutation were unresponsive versus 6 of 16 without a mutation (1 Australian patient did not undergo posture studies). This was significant (P<0.02, Fisher’s exact test), as was the change in aldosterone on standing when compared in the 2 genotypes (Figure 3 and Figure S2 in the online-only Data Supplement).

Discussion

Aldosterone plays a key role in regulating blood pressure, and K+ channels have been widely implicated in its release from zona glomerulosa (ZG) cells in the adrenal cortex. However, previous interest has focused on TASK-like K+ channels...
TWIK-like acid sensing K\(^+\) channels, such as TASK and TREK\(^{1,12}\) which belong to the 2-pore subfamily of K\(^+\) channels. These channels are only distantly related to other voltage-sensitive K\(^+\) channels or inward-rectifying channels, such as KCNJ5\(^{13}\). The 2-pore K\(^+\) channels give the ZG cell selective permeability to K\(^+\) and, hence, the ability to sense a wide range of extracellular K\(^+\) concentrations\(^{14}\). Their importance to the ZG cell has been highlighted in 2 different mouse knockout models. The first knocked out both TASK1 and TASK3 channels to produce mice with partially depolarized ZG cells and features of PA.\(^{15}\) These mice provide a model of PA reflecting bilateral adrenal hyperplasia. In the second model hyperaldosteronism was also produced by isolated knockout of the TASK1 channel, but in this model it could not be attributed to depolarization of adrenal cortical cells.\(^{16}\) In fact, the ZG cells of these mice had a normal membrane potential because of compensatory expression of other channels, such as TASK3. Nevertheless, the adrenals of these mice show abnormal zonation and ectopic expression of the aldosterone synthase enzyme CYP11B2 in the ZF cells. This ectopic pattern is reminiscent of human glucocorticoid-remediable aldosteronism where aldosterone can be synthesized in ZF cells, as well as the ZG cells, and may explain the PA in this model. So, knockout of 2-pore K\(^+\) channels can reproduce some features of human PA, but there is no evidence that these channels have a role in APA formation itself.

The discovery that a mutation in a K\(^+\) channel, KCNJ5, causes a familial form of PA (recently labeled “FH-III,” Online Mendelian Inheritance in Man ID 613677 at http://www.ncbi.nlm.nih.gov/omim) provides a novel molecular mechanism for the autonomous secretion of aldosterone\(^{5}\), but it becomes all the more important with the discovery that sporadic APAs can also have somatic mutations in the same region of the \(\text{KCNJ5}\) gene (see Reference\(^{5}\) and the present study). It is also relevant that population sequencing strategies, such as the 1000 Genomes Project, have not detected these or other functional single nucleotide polymorphisms within this same region of the \(\text{KCNJ5}\) gene in the general population. It was originally suggested that somatic mutations

![Figure 1. Representative sequencing chromatograms from mutants detected in United Kingdom aldosterone-producing adenomas (APAs) or adjacent adrenal gland tissue (AAG). The AAG or peripheral blood lymphocyte genomic DNA (gDNA) was used to confirm the somatic status of the mutations detected.](image1)

![Figure 2. Scatter plots of aldosterone-producing adenoma (APA) size (largest diameter) arranged by genotype for the combined APA collections. The horizontal lines are the group means and 95% CI. The APAs carrying a KCNJ5 somatic mutation were significantly larger than those with a wild-type (WT) sequence (*P<0.0001), but G151R and L158R APAs did not differ significantly in size.](image2)

![Figure 3. Recumbent (R) and upright (U) plasma aldosterone plotted for subjects with an aldosterone-producing adenoma (APA) carrying either a KCNJ5 mutation (mutant) or a wild-type (WT) KCNJ5 sequence. The groups are significantly different: *P<0.002.](image3)
may be restricted to large, atypical APAs (>2 cm).\(^{17}\) This possibility can now be eliminated, because we identified somatic \textit{KCNJ5} mutations in approaching half of our unselected APAs. Our collections come from opposite sides of the world and were diagnosed through a mixture of screening in the center’s own hypertension clinic and referrals of patients with possible PA from other hospitals. So ascertainment bias is unlikely, and the overlapping 95% CIs for the mutation frequency in the 2 centers show that somatic \textit{KCNJ5} mutations are common. Although the APAs carrying mutations are, on average, larger, we have identified \textit{KCNJ5} mutations in a number of tumors <1 cm in diameter. Finally, for reassurance we screened FH-II samples but found no germ-line mutations, suggesting that FH-II is genetically, as well as phenotypically, distinct from FH-III.

Somatic mutations are implicated in the pathogenesis of a number of endocrine adenomas, including \textit{GNAS1} mutations in growth hormone–secreting pituitary adenomas\(^{18}\) and activating mutations in the thyrotropin receptor gene associated with thyroid adenomas.\(^{19}\) It is also interesting that these somatic mutations often cluster around a few key amino acid residues. For example, in 40% of growth hormone–secreting adenomas somatic mutations substitute just 2 residues in \textit{GNAS1}, encoding arginine 201 (R201) or glutamine 227 (Q227).\(^{20}\) It is perhaps not surprising that such activating mutations can provide a growth stimulus by stimulating G protein–linked signaling pathways and \(c\)AMP production. Whether \textit{KCNJ5} mutations operate in a similar way as the sole cause of APAs or require a second “hit” remains to be established. In the mendelian syndrome, massive hyperplasia occurs, but it is unclear by what mechanism \textit{KCNJ5} mutations stimulate growth in an adrenocortical cell.\(^{5}\) All of the mutations identified to date, including the delI157 mutation, are located within either the selectivity filter itself or the adjacent inner vestibule of the ion channel.\(^{1}\) The G151R arginine substitution affects the first key glycine in the GYG motif that is present in almost all of the \(K^+\) channels with the notable exception of the 2-pore subfamily.\(^{21}\) The delI157 mutation will lead to closer positioning of R155 and E159 (RV1157TE) and conceivably disrupt the formation of crucial salt bridges around the pore. These salt bridges are thought to be important in sterically constraining the size of the inner mouth of the filter and, hence, controlling cation selectivity.\(^{22}\) The remaining arginine substitution (L168R) may also disrupt salt bridge formation by introducing a local positive charge. It remains to be proven, but it seems plausible that these mutations would constitutively depolarize the cells within an APA. This could explain autonomous secretion of aldosterone by an APA with depolarization-activated \(Ca^{2+}\) entry leading perhaps to calcium-triggered growth.

Ganguly et al\(^{23}\) suggested in 1973 that APAs could be distinguished by the failure of their plasma aldosterone to rise on standing after overnight recumbency. This was in marked contrast to the rise seen in normal subjects and patients with PA because of bilateral adrenal hyperplasia. It was subsequently shown that this lack of response to posture or the infusion of angiotensin (Ang) II was not shared by all of the APAs and appeared restricted to those with ZF morphology and the production of excessive “hybrid” steroids18-hydroxy- and 18-oxo-cortisol.\(^{9,10}\) This makes our observation that our APAs with mutations lacked an aldosterone response to posture all the more intriguing, because it represents the response of the APA cells to Ang II generated in vivo by the renin-Ang system on standing.\(^{9,10,24}\) Possibly, APA cells depolarized by \textit{KCNJ5} mutations cannot respond to Ang II receptor activation by further depolarization and aldosterone release. Alternatively, the histology of APAs that are unresponsive to posture and secrete excess amounts of the hybrid 18-oxo steroids may be relevant. They are made up of predominantly large lipid-laden ZF cells versus the smaller and more nucleus-dominant ZG cells,\(^{10}\) and ZF-type cells could be inherently less sensitive to Ang II than ZG cells, perhaps reflecting fewer or downregulated Ang II receptors.

This speculation is further supported by biochemical and histological genotype:phenotype analysis of the United Kingdom cohort showing that the \textit{KCNJ5} mutations are associated with APAs at the ZF end of the spectrum.\(^{25}\) Importantly, the pedigree bearing a germline \textit{KCNJ5} mutation and characterized by florid PA with marked bilateral hyperplasia of ZF also demonstrated markedly elevated serum and urinary levels of “hybrid” steroids.\(^{10}\) Understanding how these molecular signatures are related to the electrophysiological changes that follow somatic \textit{KCNJ5} mutation should lead to a better understanding of APA tumorigenesis.

In summary, we have confirmed that somatic mutations in the selectivity filter of the \(K^+\) channel \textit{KCNJ5} are common in APAs regardless of their size. Although all mutations in the selectivity filter region may have a common electrophysiological phenotype, removing selectivity for the \(K^+\) ion, evidence is accruing that the APAs carrying these mutations also have a different clinical and physiological phenotype to nonmutation-bearing APAs.\(^{25}\) Features such as a lack of aldosterone responsiveness to Ang II and production of hybrid steroids have been shown to predict preoperatively the cellular makeup of the APA.\(^{9,10}\) Further investigation is required to determine whether these same observations can predict APA genotype or whether APA genotype itself predicts prognosis.

**Perspectives**

APAs were once thought to be very uncommon causes of hypertension. It is now realized that they may account for 3% to 5% of cases of what was previously labeled as essential hypertension. This article confirms a recent report that sporadic APAs contain somatic mutations in a potassium channel that was not previously highlighted as being important in the adrenal cortex, the inward rectifying potassium channel \textit{KCNJ5}. This channel is well known outside of the adrenal and mediates, for example, slowing of the heart by the vagus nerve. We found previously reported somatic \textit{KCNJ5} mutations in 40% of a large collection of APAs (73) from 2 centers and a previously unreported deletion mutation, delI157, also in the proximity of the selectivity filter. The \textit{KCNJ5} mutations probably depolarize adrenal cortical cells, thereby stimulating aldosterone release. Interestingly, APAs with a \textit{KCNJ5} mutation have the histological, biochemical, and physiological phenotype of Ang II unresponsive tumors. Somatic mutations are surprisingly common in unselected
APAs and are not restricted to large APAs. These findings contribute significantly to our understanding of the molecular mechanisms involved in the development of the classic Conn tumor.

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Disclosures
None.

References
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Somatic mutations affecting the selectivity filter of KCNJ5 are frequent in two large unselected collections of adrenal aldosteronomas.


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The following PCR primers (5’-3’) were used to sequence the coding region of the human KCNJ5 gene:

KCNJ5_E21F  GATGGTGCTTTTTAACTCAAAGC
KCNJ5_E21R  GTGATGACTCGGAAGCCATACC
KCNJ5_E22F  CTTTCCTGTTCCTCATGAGACC
KCNJ5_E22R  CTGAGGAGGACAAAAGGCC
KCNJ5_E3F   ATGCATGTAACTTCCGTTC
KCNJ5_E3R   GCCAGTGACAGGGAGTCTTAGG
KCNJ5_mid_F CTTCATTGTGGGCTCATTC
KCNJ5_mid_R CCACCATGAAGGCATTGACG
KCNJ5_mid_R2 GGGACTTGATGAGCTTGCC

Supplemental figure, S1. Scatter plots of APA size (largest diameter) arranged by genotype and collection centre (open=Australia; filled=UK). The horizontal lines are the group means.
Supplemental figure, S2. The effect of KCNJ5 genotype on individual Δaldosterone values for the postural response of plasma aldosterone (U-R, upright – recumbent). Individuals are plotted with medians shown by the horizontal lines. The groups are significantly different: * P<0.002.