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# Mutations of the Potassium Channel KCNJ5 causing Aldosterone-Producing Adenomas: One or Two Hits?

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Primary aldosteronism (PA) is the most common cause of secondary hypertension and is associated with a significant increase in cardio- and cerebro-vascular morbidity (1). The most common forms of PA are aldosterone-producing adenomas (APA) and idiopathic hyperaldosteronism (IHA) with APA responsible for 30-50% of cases (1). Recently discovered somatic mutations of the potassium ($K^+$) channel KCNJ5 gene coding for Kir3.4, a potassium inwardly rectifying channel, subfamily 1, member 5, were postulated to cause aldosterone-producing adenomas in 8 of 22 patients (2). While crucial, the KCNJ5 mutations may not completely explain the histological and molecular findings in APAs; other events, or a 'second hit,' may be involved.

Three familial hyperaldosteronism syndromes have been described. In FH-I (glucocorticoid-remediable aldosteronism) a gene duplication from the crossover recombination of the promoter region of *CYP11B1* and most of the coding region of *CYP11B2* producing a chimeric gene expressed in the zona fasciculata (3). FH-II is defined as the presence of at least two close relatives having hyperaldosteronism (either having APA or IHA), in whom genetic testing for the hybrid gene is negative. The etiology of FH-II is unknown, though there is a linkage with chromosomal region 7p22 in some patients (3). FH-III was described in single affected family in which all three members had severe hypertension and very high levels of aldosterone and the hybrid steroids 18-hydroxycortisol and 18-oxocortisol that were not glucocorticoid-suppressible (4) and required bilateral adrenalectomy to control the hypertension and hyperaldosteronism. The adrenals were very large with massive hyperplasia and hypertrophy of cells with histological features of adrenal fasciculata or transitional zone cells (4). This family was found to have a germinal mutation in the $K^+$-selectivity filter sequence of the *KCNJ5* gene, T158A, (2).

The aldosterone secretagogues angiotensin II, $K^+$ or ACTH induce adrenal zona glomerulosa cell membrane depolarization through the activation of $K^+$ channels, resulting in opening of calcium channels and stimulation of calcium-activated signal transduction pathways, increasing expression of enzymes of aldosterone synthesis, thereby increasing aldosterone (2, 5). Two somatic mutations in the $K^+$-selectivity filter sequence of the *KCNJ5* gene, G151R and L168R, were present in approximately a third of APA patients (2). These

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3 mutations decrease $K^+$ channel specificity, increase $Na^+$ conductance and membrane depolarization, resulting in loss of control of aldosterone synthesis (2).

Mulatero *et al*(6) reports results of a search for *KCNJ5* mutations in 46 patients from 21 FH-II families. A new germline mutation, G151E, was identified in two related patients which was absent in 7 unaffected relatives. In addition, 3 somatic mutations were identified in adenomas from this cohort, G151R, L168R as previously described and T158A, the germline mutation found in the original FH-III family (2). The phenotype of the patient with the T158A somatic mutation was similar to other patients in this cohort with APA and milder than that of the FH-III patients with the T158A germline mutation. This study demonstrates that the definition of FH-II is too broad and comprises multiple etiologies.

The hypertension, hypokalemia and hyperaldosteronism of the 2 patients with the newly described KCNJ5 mutation, G151E, were mild to moderate and easily controlled with low doses of mineralocorticoid receptor antagonists. In one, levels of 18-hydroxycortisol and 18-oxocortisol were normal (6); in the other they were within the range of patients with APA, but significantly lower than patients with FH-I and in the FH-III family with germline T158A mutation (4). Adrenal glands of the patients with the KCNJ5 G151E mutation were normal by CT scan. The electrophysiological characteristics of cells transfected with the $KCNJ5^{G151E}$ cDNA were similar to those of cells transfected with the G151R and L168R (2). Thus, FH-III caused by germline mutations of the *KCNJ5* gene selectivity filter has variable phenotypes, whether due to the different mutation itself, or as yet unknown factors.

Relatively few APAs have been sequenced, yet the frequency of somatic mutations of the KCNJ5 channel is very high, suggesting that this is a hot area for mutations and more may be discovered. The reason for frequent somatic mutations in APA is unclear. Germline mutations the *KCNJ5* gene are rare and were not found in an analysis of 1,000 genomes (2).

In rats the zona glomerulosa ZG is distinct and forms a continuous zone of 3 to 10 cells wide depending on sodium intake. The unique enzyme in the synthesis of aldosterone, cyp11b2, is expressed only in this zone and the number of cells expressing cyp11b2 increases with chronic sodium depletion (7). A chronically high sodium diet decreases the number of cells expressing the cyp11b2 in the rat ZG, but nests of cells strongly expressing it remain (7). The human adrenal exhibits areas of a variegated zonation with the ZG comprising small cells in a discontinuous pattern and subcapsular aldosterone-producing cell clusters (APCCs) expressing the CYP11B2 enzyme (8, 9). It is likely that the discontinuous APCC pattern is analogous to that of a rat on a high sodium diet, as most normal human adrenals are obtained from patients undergoing nephrectomy for renal cancer who are on a standard, relatively high sodium diet. This pattern of CYP11B2 expression suggests that aldosterone biosynthesis is regulated by the renin-angiotensin-aldosterone system in the regular and “discontinuous” ZG, while aldosterone production may be autonomous in the APCCs (8) which maybe analogous to the nests of cells expressing cyp11b2 in the ZG of rats on a high sodium diet (7).

The histopathology of APAs is complex. A large proportion of patients have peri-tumoral zona glomerulosa hyperplasia and sometimes micronodules in addition to the adenoma (9). In most patients, in addition to expression in the APA, CYP11B2 is frequently expressed in APCCs distant to the adenoma (8, 10). Depending on the patient, CYP11B2 expression varies from virtually 100% to only 40% of the cells in the APA (8). CYP11B1 enzyme expression in APAs varies between just a few to 20% of the cells, however it appears that CYP11B1 and CYP11B2 are not expressed in the same cell and within adenomas there are cells that express neither enzyme. 17α- hydroxylase is also expressed only in the cells expressing CYP11B1 (8). APCCs are frequently found in adrenals with cortisol-producing

adenomas in which the zona fasciculata and most of the ZG are atrophic (8, 10). These APCCs appear to function autonomously (8), but this remains to be proven.

In the original description of FH-III (2), the KCNJ5 mutations comprised 33 and 29% of the reads in the exome sequencing used to detect mutations. It is not known if the KCNJ5 mutations occur in all the cells within an adenoma (2, 6). In addition, whether the KCNJ5 mutations are responsible for increased cell proliferation resulting in adenoma formation is unclear (8, 9). Genes linked to adrenal stem/precursor cells and to nuclear receptors that have a significant role in adrenal development were recently described for normal adrenals, as well as APAs and the peri-tumoral area. These include Sonic hedgehog (Shh), β-catenin, CD56, steroidogenic factor 1, and dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome, gene 1 (DAX-1) (10). While Shh is expressed in only a few cells beneath normal adrenal capsules, it is very highly expressed in the entire APA and hyperplastic peri-tumoral ZG. Wnt/β-catenin signaling is also activated in both the APA and peri-tumoral cortex. It is tempting to hypothesize that mutations of the KCNJ5 gene occur relatively frequently in the activated adrenal stem cell/progenitor cells, causing the autonomous production of aldosterone and primary aldosteronism. The hyperplastic zona glomerulosa and APCCs found at a distance from the adenoma suggest that the initial yet unidentified event stimulate adrenal stem cell/precursors, followed by a somatic mutation in one of these stimulated cells, causing excessive production of aldosterone in addition to proliferation. This might explain the lack of suppression of aldosterone production by the contralateral adrenal, a very common finding of adrenal vein sampling to diagnose APA. One can postulate that adenomas are caused by a second event within a hyperplastic zona glomerulosa. It will be interesting to know whether suppression of aldosterone synthesis by the contralateral adrenal occurs in patients with an APA with a KCNJ5 mutation. Many APA may represent a toxic adenoma within a hyperplastic adrenal zona glomerulosa, analogous to a toxic nodule within a multinodular goiter, with two ‘hits’ required to develop the APA.

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