KCNJ5 Mutations: Sex, Salt and Selection

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Key words
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- primary aldosteronism
- *KCNJ5*
- potassium channel

Abstract

Somatic mutations have been identified in the *KCNJ5* gene (encoding the potassium channel GIRK4) in aldosterone-producing adenomas (APA). Most of these mutations are located in or near the selectivity filter of the GIRK4 channel pore and several have been shown to lead to the constitutive overproduction of aldosterone. *KCNJ5* mutations in APA are more frequent in women; however, this gender dimorphism is a reported phenomenon of Western but not East Asian populations. In this review we discuss some of the issues that could potentially underlie this observation.

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Introduction

The original description of somatic aldosterone-producing adenoma (APA) mutations in the *KCNJ5* gene by Choi et al. in 2011 [1] marks one of the defining moments in primary aldosteronism (PA) research that initiated an extraordinary avalanche of studies in this field. Within 2 years of the discovery of *KCNJ5* mutations in APAs, the application of next-generation sequencing methods led to the identification of somatic APA mutations in 3 other genes. The affected genes were *ATP1A1* and *ATP2B3*, encoding Na^+/K^+-ATPase 1 and Ca^{2+}-ATPase 3, respectively, and *CACNA1D* that encodes a subunit of an L-type voltage gated calcium channel, Cav1.3 [2–4]. However, in an intriguing development reported at the symposium *Progress in Primary Aldosteronism 4* held in June 2015 and published recently [5], somatic mutations in *ATP1A1*, *ATP2B3* and *CACNA1D* have also been identified in aldosterone-producing cell clusters (APCCs) in normal adrenal glands that stimulate *CYP11B2* activity and aldosterone production. In a large European collaboration, Fernandes-Rosa et al. [6] reported the combined prevalence of somatic mutations in APAs in *KCNJ5*, *ATP1A1*, *ATP2B3* and *CACNA1D* as 54% (**Fig. 1**). The functional effect of the thus far described somatic APA mutations all converge at the same intracellular level of the Ca^{2+} signalling pathway that ultimately lead to an increase in aldosterone biosynthesis [7–10]. The original identification of the somatic APA mutations in *KCNJ5* [1] and the combined efforts of several other groups highlighting the role of these mutations in unilateral PA in driving aldosterone excess is surely one of the major advances in hypertension research over the last decade.

G Protein-Activated Inwardly-Rectifying Potassium Channels

GIRK4 (encoded by *KCNJ5*, cytogenetic location: 11q24.3) is a member of the family of G protein-activated inwardly-rectifying potassium channels and is expressed at the plasma membrane in various different tissues, including the heart, central and peripheral neurons, various endocrine tissues, as well as in non-excitable structures [11]. GIRK channels form transmembrane permeation pathways or pores with a high selectivity for K^+ thereby preferentially allowing K^+ to flow into the cell. However, the directional flow of K^+ depends on where the ion channel is expressed. In adrenal zona glomerulosa cells, GIRK channels participate in maintaining the hyperpolarised state of the cell along with other K^+ channels by allowing an outward flow of K^+ conductance [12]. The K^+ ion gradient across the cell membrane in GIRK channels is modulated in a voltage-dependent manner by the occlusion of the channel pore by intracellular polyamines and Mg^{2+} [13]. A GIRK channel subunit consists of 2 transmembrane helices with a large cytoplasmic domain and the N and C termini extruding into

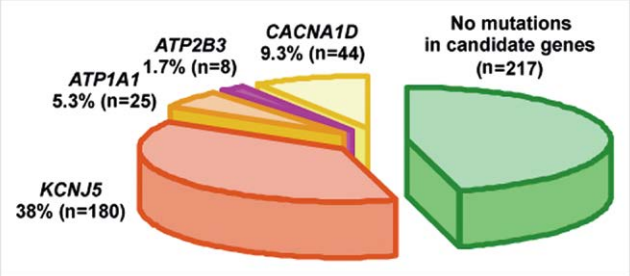


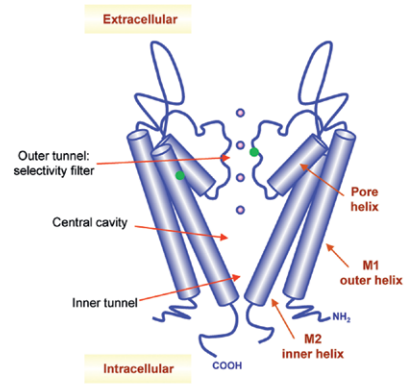
Fig. 1 Prevalence of somatic mutations in APA reported in a European cohort: The prevalence of somatic APA mutations in *KCNJ5*, *ATP1A1*, *ATP2B3* and *CACNA1D* reported in a European cohort of APA (n = 474) recruited through the ENS@T registry (European Network for Adrenal Tumors, http://www.ensat.org/) [6].

the cytosol. An extracellular loop forms the selectivity filter that comprises the highly conserved signature sequence Gly-Tyr-Gly of K^+ channels. Functional GIRK channels are composed of 4 homo- or heterotetrameric subunits. Although GIRK4 can be expressed as a homotetramer, in most tissues and cell types it is found as a heterotetrameric complex with GIRK1, GIRK2 or GIRK3 [12].

The activation of GIRK channels is via direct interaction of the cytoplasmic domains with the G protein βγ subunit and this process may also involve the anionic lipid phosphatidylinositol 4,5-bisphosphate [14]. In contrast, the inactivation of GIRK4 appears to be mediated by phospholipase C activation [15]. The crystal structure of GIRK4 has not been resolved, however, that of the related channel GIRK2 in the closed as well as a constitutively active conformation has been determined both in the absence and presence of phosphatidylinositol 4,5-bisphosphate providing insights into the molecular mechanisms of the multi-ligand regulation of GIRK channels [16].

Somatic *KCNJ5* Mutations in APA

Choi et al. [1] identified GIRK4-p.Gly151Arg and p.Leu168Arg substitutions in 8 out of 22 APAs. Both of these mutations interfere with the selectivity filter of the ion channel and lead to the indiscriminate conductance of Na^+ through the pore of the outer tunnel (**Fig. 2**). The resultant membrane depolarisation causes the opening of Ca^{2+} voltage-gated ion channels, Ca^{2+} influx, activation of *CYP11B2* transcription and increased aldosterone biosynthesis. The ensuing renewed interest in PA research led to the rapid determination of the prevalence of somatic *KCNJ5* mutations in several cohorts of APAs [6, 17–29]. Foremost was the study by Boulkroun et al. [17] (**Table 1**) that additionally reported the predominance of APA-*KCNJ5* mutations in women, an observation that was subsequently corroborated by others (**Table 1**). Several novel mutations in *KCNJ5* associated with APAs were also identified (**Fig. 3**), however, the mutations that were first described are by far the most prevalent [1]. A recent meta-analysis that incorporated 13 studies involving 1636 patients showed that female gender is a phenotype of APA patients carrying *KCNJ5* mutations along with pronounced aldosteronism, larger tumour size and younger age [30].



Mutation	Reference	Mutation	Reference
Gly151Arg	Choi 2011 [1]	Glu246Gly	Cheng 2015 [22]
Leu168Arg	Choi 2011 [1]	Ala139-Phe142dup	Xu 2015 [26]
delIle157	Azizan 2012 [18]	Gly153-Gly164dup	Wang 2015 [28]
Glu145Gln	Åkerström 2012 [19]	Thr148-Thr149insArg	Zheng 2015 [29]
Thr158Ala	Mulatero 2012 [20]	Ile157Lys	Scholl 2015 [53]
Trp126Arg	Williams 2014 [10]	Phe154Cys	Scholl 2015 [53]
insThr149	Kuppusamy 2014 [24]	Ile150_Gly151insMet	Scholl 2015 [53]
Arg115Trp	Cheng 2015 [22]	Ile144_Glu145insAlalle	Scholl 2015 [53]

Fig. 2 Cartoon of the pore module of GIRK4: The cartoon represents the pore module of a G protein inwardly rectifying K^+ channel. The first transmembrane segment M1 faces the lipid bilayer and the second transmembrane segment forms the inner pore helix. The K^+ permeation pathway comprises the narrow outer tunnel with the ion selectivity filter, the central cavity and the inner tunnel. Functional GIRK channels are composed of 4 homo- or heterotetrameric subunits and GIRK4 exists both as a homotetramer but more usually as a heterotetramer. The approximate positions of p.Gly151Arg in the selectivity filter of the outer tunnel and p.Leu168Arg in the inner helix are shown as green spheres. The somatic mutations identified to date with the reference that first reported them are shown in the panel below the figure. The figure was modified from a schematic diagram of the pore module of the *Streptomyces lividans* K^+ channel [54] according to the corresponding structure of the chicken KCNJ12 channel [1], that shares 89% identity with human GIRK4 within the pore region and selectivity filter.

Reported Prevalence of *KCNJ5* Mutations in APA Patients in Different Populations

Unilateral PA patients from Western populations report a lower prevalence of somatic APA *KCNJ5* mutations compared to East Asian populations (**Table 1**, **2**, **Fig. 3**, 39 vs. 73%, $p < 0.0001$). Bilateral adrenal hyperplasia (BAH or idiopathic aldosteronism) is generally reported to account for around 2-thirds of all cases of PA [31]. In contrast, a Japanese nationwide epidemiological study reported that the frequency of unilateral APA predominated over BAH accounting for 86% of cases of APA and 14% BAH patients (n = 1409) [32]. It is likely that this indicates a selection bias for APA patients that display a more pronounced phenotype with higher blood pressure levels compared to BAH patients; in fact, in the study by Miyake et al. [32], the unilateral APA patients displayed higher blood pressure levels compared to the BAH patients. Further, in China, where accessibility to AVS centres is limited, a selection bias is likely to exist and favour patients with a more florid phenotype potentially meaning those with large adrenal nodules. Other factors influencing the selection bias for APA in Japanese patients could be the use of ACTH stimulation during adrenal venous sampling (AVS) for subtype diagnosis in all Japanese centres: this could theoretically increase the aldos-

terone production from the adenoma, thereby enhancing the probability of detecting an APA [33]. Also, APA carrying *KCNJ5* mutations are more frequently composed of zona fasciculata-like cells; in contrast to APA of "wild type" status, or of APA carrying *ATP1A1*, *ATP2B3* or *CACNA1D* mutations, that are comprised principally of zona glomerulosa-like cells [23, 34, 35]. Because ACTH is the physiological regulator of hormone production in the zona fasciculata, whereas angiotensin II and K^+ mainly control hormone production in the zona glomerulosa, the use of ACTH stimulation during AVS in Japanese centres may also introduce a selection bias for APA carrying *KCNJ5* mutations. Therefore, these factors could contribute to the disproportionate selection of the APA subtype and potentially of *KCNJ5*-mutated APAs in Japanese populations compared to Western populations. In an interesting development, a recent meta-analysis demonstrated a correlation between mean daily urinary Na^+ excretion with the frequency of *KCNJ5* mutations in APA and hypothesised that higher average dietary Na^+ intake may cause a more severe phenotype and/or earlier detection of the disease in APA patients [30].

The INTERMAP population study illustrated the wide variations in salt consumption between East Asian (China and Japan) and Western (USA and UK) diets in which participants from East Asia on a self-reported reduced salt diet displayed higher daily urinary Na^+ excretion compared with Western participants on a non-reduced salt diet [36] (**Table 3**). Further, the Intersalt international study of electrolyte excretion and blood pressure from 52 centres, with a Na^+ excretion range of 0.2 mmol/24 h (Yanomamo Indians, Brazil) to 242 mmol/24 h (North China), a significant positive association between daily urinary Na^+ excretion and systolic blood pressure and between the Na^+/K^+ ratio and systolic blood pressure was found [37]. Four of the 52 centres were isolated populations that displayed very low sodium excretion, low sodium/potassium excretion, low body mass index, and low alcohol consumption. In these populations, high blood pressure was rare or non-existent [37, 38].

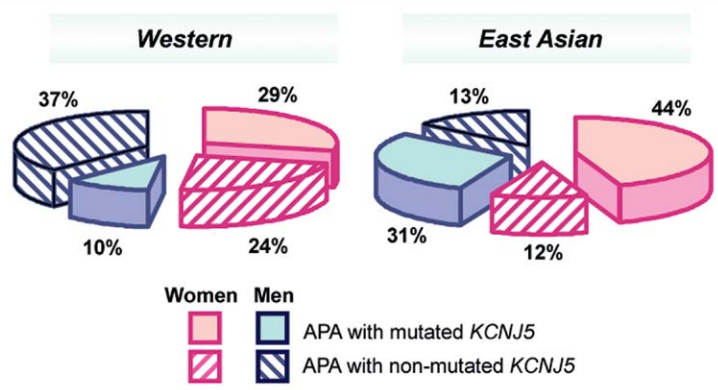
In a global survey carried out in 2010, mean Na^+ intake was 3.95 g/day, twice the WHO recommended limit (2 g Na^+/day). Intakes were highest in East Asia, Central Asia and Eastern Europe (mean > 4.2 g/day). In contrast, regional mean intakes in North America, Western Europe and Australia/New Zealand ranged from 3.4 to 3.8 g/day [39].

Table 1 Reported prevalence of *KCNJ5* mutations according to gender in Western populations.

Population	Sample size	*KCNJ5* mutations associated with unilateral PA	Reference
West Europe	**380**	**129/380 (34%)**	Boulkroun 2012 [17]
Men-No. (%)	182/380 (48%)	32/182 (19%)	
Women-No. (%)	198/380 (52%)	97/198 (49%)	
West Europe-Australia	**348**	**157/348 (47%)**	Åkerström 2012 [19]
Men-No. (%)	151/348 (43%)	35/151 (23%)	
Women-No. (%)	197/348 (57%)	122/197 (62%)	
USA-Italy	**42**	**13/42 (31%)**	Monticone 2012 [9]
Men-No. (%)	18/42 (43%)	3/18 (17%)	
Women-No. (%)	24/42 (57%)	10/24 (42%)	
Norway	**28**	**10/28 (36%)**	Arnesen 2013 [21]
Men-No. (%)	20/28 (71%)	5/20 (25%)	
Women-No. (%)	8/28 (29%)	5/8 (63%)	
Italy	**112#**	**44/112 (39%)**	Williams 2014 [10]
Men-No. (%)	58/112 (52%)	17/58 (29%)	
Women-No. (%)	54/112 (48%)	27/54 (50%)	
The Netherlands	**39 ***	**22/39 (56%)**	Dekkers 2014 [23]
Men-No. (%)	18/39 (46%)	7/18 (39%)	
Women-No. (%)	21/39 (54%)	15/21 (71%)	

* There were 53 samples in the original study but of these 39 samples had complete information for gender and genotype which are included here

#Thirty-three of these samples were included in the study of Boulkroun [17] and were excluded from the subsequent analysis depicted in **Fig. 3**. These 33 samples comprised 16 women with 10 APA carrying *KCNJ5* mutations and 17 men with 5 APA carrying *KCNJ5* mutations



Population	Sample size	*KCNJ5* mutations associated with unilateral PA		
Western	**916**	**360/916 (39%)**		
Men - No. (%)	430/916 (47%)	*p*<0.0001	94/430 (22%)	*p*<0.0001
Women - No. (%)	486/916 (53%)		266/486 (55%)	
East Asian	**413**	**310/414 (73%)**		
Men - No. (%)	184/413 (45%)		130/184 (71%)	*ns*
Women - No. (%)	229/413 (55%)		180/229 (79%)	

Fig. 3 Apparent prevalence of *KCNJ5* mutations according to gender and region: The Western populations group comprises Western European countries, Australia and the USA; the East Asia group comprises China and Japan. The pie charts represent the percentage of men or women with or without APA carrying *KCNJ5* mutations as a function of the total number of APA (men + women). The panel under the figure gives the percentage of men or women with APA carrying *KCNJ5* mutations as a function of the number of APA from the same gender as indicated. P-values were calculated using the Yates Chi-squared test and $p < 0.05$ was considered significant. *ns*: Not significant. The frequencies of *KCNJ5* mutations in APA in the men vs. men and women vs. women from the Western populations compared to the Eastern Asia groups were also significantly different ($p < 0.0001$).

Table 2 Reported prevalence of *KCNJ5* mutations according to gender in East Asian populations.

Population	Sample size	*KCNJ5* mutations associated with unilateral PA	Reference
Japan	**5**	**5 (100%)**	Monticone 2012 [9]
Men-No. (%)	2/5 (40%)	2/5 (100%)	
Women-No. (%)	3/5 (60%)	3/5 (100%)	
Japan	**23**	**15/23 (65%)**	Taguchi 2012 [25]
Men-No. (%)	10/23 (43%)	7/10 (70%)	
Women-No. (%)	13/23 (57%)	8/13 (62%)	
Japan	**108** *	**75/108 (69%)**	Kitamoto 2015 [27]
Men-No. (%)	37/103 (36%)	25/37 (68%)	
Women-No. (%)	66/103 (64%)	50/66 (76%)	
China	**114**	**86/114 (75%)**	Wang 2015 [28]
Men-No. (%)	52/114 (46%)	40/52 (77%)	
Women-No. (%)	62/114 (54%)	46/62 (74%)	
China	**168**	**129/168 (77%)**	Zheng 2015 [29]
Men-No. (%)	83/168 (49%)	56/83 (67%)	
Women-No. (%)	85/168 (51%)	73/85 (86%)	

* The original study comprised 108 patients and excluded 5 of them from their analysis due mutations in other genes (*ATP1A1*, *ATP2B3*, *CACNA1D*)

Table 3 Daily urinary Na^+ excretion in all participants from the INTERMAP population study reporting non-reduced salt and reduced salt diets.

Nation	Non-RSD	RSD
UK	144.7 ± 2.0 (n = 447)	155.2 ± 9.0 (n = 24)
USA	163.6 ± 1.2 (n = 2036)	148.9 ± 4.2 (n = 159)
Japan	198.9 ± 1.5 (n = 1109)	181.0 ± 8.4 (n = 36)
China	228.3 ± 2.6 (n = 828)	171.5 ± 22.5 (n = 22.5)

Data were from the study by Okuda et al. [36]. Numbers refer to the 24-h urinary Na^+ excretion in mmol (mean ± standard error). In parenthesis, n = number of participants. RSD: reduced salt diet; non-RSD, Non-reduced salt diet

According to the China national diabetes and metabolic disorders study, 26.6% of Chinese adults (20 years or older, approximately 254 million individuals), are hypertensive [40]. If dietary salt intake is correlated to a more severe phenotype and/or earlier detection of the disease in APA patients [30] then it is likely to have a more pronounced effect in East Asian patients. Finally, another factor that could influence the detection of PA and some of its subtypes in different populations, is the difference in blood pressure sensitivity to aldosterone among races [41] that could potentially affect phenotype, detection and referral in different ethnic groups. However, the relevance of this in East Asians compared to other ethnic groups is unknown.

Increased Prevalence of *KCNJ5* Mutations in APA in Western Women

KCNJ5 mutations in APA have consistently been reported as more frequent in women in Western populations (**Table 1**, **Fig. 3**). **Table 1** shows the prevalence of *KCNJ5* mutations in APA patients according to gender in cohorts from Europe, Australia and the USA (Western populations, **Table 1**, **Fig. 3**) with an overall predominance in women compared to men (55 vs. 22%, $p < 0.0001$), in agreement with a recent meta-analysis [30]. In contrast, this distinction was not evident in East Asia (China and Japan, **Table 2**) where there was no significant difference between genders (women vs. men: 79 vs. 71%, **Fig. 3**).

In a European population, in addition to being more prevalent in women, *KCNJ5* mutation status in APA patients was correlated with a younger age at diagnosis [6, 17]. It is tempting to speculate that the younger age at diagnosis may have been a gender effect, potentially related to the reluctance of men to seek medical help and failing to do so until the disease has progressed [42]. Consistent with this idea is a Danish study that demonstrated a lower contact rate of men with the general practitioner, and higher rates of hospitalisation and mortality [43]. It would be relevant to study this hypothesis by analysing the severity of hypertension, and associated target organ damage at study entry according to gender and genotype. Interestingly, in a study to assess trends in hypertension in US adults (NHANES 1988–1994 and 1999–2004), among the race/ethnic groups studied, women generally showed better awareness, treatment, and control rates than men [44].

The interrelation of blood pressure and sex steroids may also play a role in the gender dimorphism of *KCNJ5* mutation status in APA patients. Blood pressure levels are consistently reported to be higher in males compared to females [44–47]. This gender difference in blood pressure appears during adolescence and the relative prevalence of hypertension shifts to predominate in females in the elderly [48]. Interestingly, during the menopause transition, estradiol levels are 20% lower for Chinese and Japanese women compared to Caucasian, Hispanic or African-American women [49]. Estrogens are associated with protective cardiovascular effects [50] and 17β-estradiol inhibits aldosterone synthesis in vitro via the estrogen β receptor [51]. The role of dietary Na^+ excess in the pathogenesis of primary hypertension may have a more pronounced effect in men than in women [52]. Further, in a global survey, mean dietary Na^+ intake in men was around 10% higher than in women [39].

Therefore, pre-menopausal Western women with PA may not be exposed to the deleterious effects of higher blood pressure and higher salt intake to the extent of their male counterparts. Further, they might benefit from the protective vascular effects of estrogen. It is attractive to hypothesise that these factors may contribute to shift the balance towards *KCNJ5* mutated APAs which are associated with a more severe phenotype and a higher likelihood of coming to medical attention.

Summary and Conclusions

Patients with APA harbouring *KCNJ5* mutations display a more severe form of aldosteronism and this may cause a selection bias in East Asian populations theoretically further exacerbated in Japanese centres by the use of ACTH stimulation during AVS.

Against a background of lower blood pressure levels, lower salt intake and vascular protection by estrogens, pre-menopausal female APA patients from Western populations may present for medical examination with a more pronounced aldosteronism compared to their male counterparts. This could potentially contribute to the sexual dimorphism of APA-*KCNJ5* mutations in Western populations. In contrast, East Asian women have a higher dietary salt intake, higher blood pressure levels and lower estrogen levels than Western women.

To address any potential pathophysiological basis of these issues: 1) cohorts of APA-*KCNJ5* patients could be analysed according to clinical phenotype (blood pressure, biochemical and hormonal parameters) and gender; 2) the true prevalence of *KCNJ5* mutations could be analysed in a large (unbiased) autopsy study from

both Western and Asian populations within the same age range as the published cohort series; and 3) the mutational status of APA patients could be correlated with parameters of quality of life and psychological well-being in a multivariate adjusted analysis to study gender-dependent effects on hypertension presentation.

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Conflict of Interest

The authors declare no conflict of interest.

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