Primary aldosteronism (PA) is the most frequent cause of endocrine hypertension. Three forms of familial hyperaldosteronism (FH) have been described, named FH-I to -III. Recently, a mutation of KCNJ5 has been shown to be associated with FH-III, whereas the cause of FH-II is still unknown. In this study we searched for mutations in KCNJ5 in 46 patients from 21 families with FH, in which FH-I was excluded. We identified a new germline G151E mutation in 2 primary aldosteronism–affected subjects from an Italian family and 3 somatic mutations in aldosterone-producing adenomas, T158A described previously as a germline mutation associated with FH-III, and G151R and L168R both described as somatic mutations in aldosterone-producing adenoma. The phenotype of the family with the G151E mutation was remarkably milder compared with the previously described American family, in terms of both clinical and biochemical parameters. Furthermore, patients with somatic KCNJ5 mutations displayed a phenotype indistinguishable from that of sporadic primary aldosteronism. The functional characterization of the effects of the G151E mutation in vitro showed a profound alteration of the channel function, with loss of K⁺ selectivity, Na⁺ influx, and membrane depolarization. These alterations have been postulated to be responsible for voltage gate Ca²⁺ channel activation, increase in cytosolic calcium, and stimulation of aldosterone production and adrenal cell proliferation. In conclusion, we describe herein a new mutation in the KCNJ5 potassium channel associated with FH-III, responsible for marked alterations of channel function but associated with a mild clinical and hormonal phenotype. (Hypertension. 2012;59:235-240.) • Online Data Supplement

Key Words: familial hyperaldosteronism ■ endocrine hypertension ■ primary aldosteronism ■ aldosterone ■ KCNJ5
FH-II is a nonglucocorticoid-remediable form of PA, phenotypically indistinguishable from sporadic PA. The molecular basis of FH-II is still unknown, although a linkage with the chromosomal region 7p22 has been shown in some, but not all, FH-II families.6,10,11 FH-II diagnosis is made when PA diagnosis is confirmed in 2 members of the same family. Recently, the genetic cause of FH-III has been unraveled: this form is attributed to a mutation in the KCNJ5 family. Recently, the genetic cause of FH-III has been described in detail elsewhere.13–15 Diagnosis of FH-II was made as occurring bilateral adrenalectomy. A characteristic feature of this family was the presence of particularly high levels of 18-hydroxycortisol and 18 oxo-cortisol and a paradoxical increase of aldosterone levels during dexamethasone-suppression testing (DST).7

The aim of our study was to search for the presence of mutations in KCNJ5 in a sample of 21 European families with FH in which the presence of the chimeric gene responsible for FH-I/GRA was excluded.

Methods
An expanded Methods section is available in the online Data Supplement at http://hyper.ahajournals.org.

Diagnosis of PA and FH and Exclusion of FH-I/GRA

Torino
Diagnosis of PA and exclusion of FH-I/GRA were performed in detail elsewhere.13–15 Diagnosis of FH-II was made as described in detail in the Primary Aldosteronism in Torino-Genetic Forms Study.16 A DST was performed only in patients carrying mutations in KCNJ5, as described previously.17

Paris
Diagnosis of PA was performed as described in detail elsewhere.18,19

Munich and Würzburg
The diagnosis of PA was established as published previously.20

Sequencing

DNA and RNA Isolation and RT-PCR
Genomic DNA was extracted from 46 patients with FH (27 from Torino, 10 from Paris, 5 from Munich, and 4 from Würzburg) and from 79 unaffected relatives (60 from Torino, 2 from Würzburg, 13 from Munich, and 4 from Paris). DNA or RNA was extracted from a total of 4 APAs (1 from Torino, 1 from Paris, and 2 from Würzburg).

KCNJ5 Sequencing
Sequencing primers were as described previously.12

Functional Study of the G151E Mutation
For functional expression in mammalian cells, specific cDNAs of human KCNJ3, KCNJ5, and mutant KCNJ5G151E were purchased from Invitrogen/Geneart and subcloned into the pIRES-CD8 expression vector.21

Results

Identification of a Family Carrying a New Germline KCNJ5 Mutation and 3 Subjects With Somatic Mutations
Twenty-seven PA patients (2 APA, 16 bilateral adrenal hyperplasia [BAH], and 9 undetermined) from 12 families identified in the Primary Aldosteronism in Torino-Genetic Forms Study16 were studied from Torino (60 unaffected relatives were available as controls). Ten PA patients (5 APA, 4 BAH, and 1 undetermined) from 5 families were studied from Paris (4 unaffected relatives were available as controls). Five PA patients (2 APA, 2 BAH, and 1 undetermined; 4 of 5 underwent adrenal vein sampling) from 2 families were studied from Munich (11 unaffected relatives were available as controls). Four PA patients (2 APA, 1 BAH, and 1 undetermined) from 2 families were studied from Würzburg (2 unaffected relatives were available as controls). Clinical

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<th>Munich + Würzburg Families</th>
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<td>5/4/1</td>
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SBP/DBP indicates systolic/diastolic blood pressure; sK+, serum potassium; PRA, plasma renin activity; DAR, direct active renin; APA, aldosterone-producing adenoma; BAH, bilateral adrenal hyperplasia; UND, primary aldosteronism with undetermined subtype; DST, dexamethasone-suppression testing. To convert aldosterone to nmol/L multiply by 0.0277; to convert PRA to ng·L⁻¹·s⁻¹ multiply by 0.2778; to convert DAR to pmol/L multiply by 0.0237. Data are expressed as mean±SD for normally distributed parameters and as median (25th to 75th percentile) for the other parameters.
and biochemical parameters of PA patients are described in Table 1.

From a total of 21 families with FH we identified a novel heterozygous germline KCNJ5 mutation (NM000890.3: c.452G/H11022A [p.Gly151Glu]) in the 2 affected members of a single family (family 10 from Torino; Figure 1), which leads to the substitution of a glycine by glutamic acid. The mutation was absent in 7 investigated unaffected relatives (Figure 2A). We also found 3 somatic APA mutations, 1 in a patient from Würzburg carrying the mutation c.451G>C/Gly151Arg and 1 in a patient from Paris carrying the mutation c.503T>G/p.Leu168Arg, both already described in APA patients,12 and a third in a patient from Torino (family 7) carrying mutation c.472A>G/Thr158Ala (Figure 2B). The latter mutation was described previously as a genomic mutation in the American family with FH-III.7,12 In all of the cases the mutations were absent in germline DNA from peripheral blood (Figure 2B).

**Phenotype of the Family With FH-III**

The index case is a white female, born in 1976. She had a history of polyuria in the first decade of life that disappeared during adolescence. She was found hypertensive in 1994 (18 years old); she was first seen in the Torino unit in 2003 when she was diagnosed with PA; she presented with high blood pressure (systolic/diastolic blood pressure: 190/115 mm Hg under doxazosin 4 mg/amlodipine 10 mg) and hypokalemia.

**Figure 1.** Family tree of the familial hyperaldosteronism (FH)-III family. Arrow indicates the index case. Black symbols indicate primary aldosteronism (PA) patients; circle with vertical lines indicate the mother of the index case, who was hypertensive since she was 45 years old and had a stroke at 70 years old. She died of cancer at 73 years of age. ALDO indicates aldosterone in ng·dL\(^{-1}\); PRA, plasma renin activity in ng·mL\(^{-1}\)·h\(^{-1}\); ARR, ALDO/PRA ratio.

**Figure 2.** Mutations identified in patients with primary aldosteronism (PA). A, DNA sequencing showing the germline KCNJ5 mutation G151E found in the 2 patients affected by PA in the familial hyperaldosteronism (FH)-III family and a normal sequence in 1 of the relatives. B, Chromatograms showing the 3 somatic KCNJ5 mutations G151R, L168R, and T158A in aldosterone-producing adenomas (APAs) from the 3 PA patients from Würzburg, Paris, and Torino with apparent familial hyperaldosteronism described in the text and the paired normal sequences obtained on peripheral DNA.
Phenotype of the 3 Subjects With Somatic Mutations in KCNJ5

The patient from Torino (family 7) carrying the T158A somatic mutation is a male born in 1954. Diagnosis of hypertension was made in 1998 (45 years old); at this time potassium was normal (4.4 meq/L), PRA was 0.3 ng/mL per hour, and aldosterone was 7.5 ng/dL. Blood pressure was 150/95 mm Hg when untreated and 135/85 mm Hg under fosinopril 20 mg. In 2002 (48 years old) he was referred to our unit for unprovoked mild hypokalemia, and diagnosis of PA attributed to a solitary APA was made (Table 2) by ARR, saline infusion, CT scanning, adrenal vein sampling, and postsurgery evaluation. Hybrid steroid levels were high but still in the range of patients with APA and much lower than FH-I/GRA and FH-III patients.

The patients from Paris and Würzburg carrying the L168R and G151R mutations, respectively, displayed phenotypes similar to those described previously by Choi et al.12 (Table 2). Clinical and hormonal parameters after adrenalectomy are described in Table S1 (available in the online Data Supplement).

Functional Effects of the G151E Mutation In Vitro

KCNJ5 is an inwardly rectifying potassium channel that can associate with other protein, for example, KCNJ3, to form heteromorphic channels.12 The G151E mutation profoundly changed the ion selectivity of the KCNJ5 potassium channel. HEK cells cotransfected with KCNJ3 and wild-type KCNJ5 were hyperpolarized under control conditions (−52±6 mV [n=8] versus −42±4 mV [n=10] in empty vector transfected cells) and exhibited a strongly inward rectifying K+ conductance. This inward current was strongly augmented by increases in extracellular K+ (Figure S1A and S1C). Ba2+ (5 mmol/L) strongly inhibited the inward whole cell current of transfected cells by 68%. In cells cotransfected with KCNJ3 and KCNJ5G151E, the membrane under control conditions was strongly depolarized to −18±1 mV (n=9). The whole cell current showed mild inward rectification, but it was unchanged when extracellular Na+ was exchanged by (serum potassium: 2.9 under potassium supplementation). Other clinical and hormonal parameters are summarized in Table 2. Long-PCR test for FH-I/GRA was negative, and hybrid steroid levels were within the normal range (Table 2) for patients with sporadic PA.16 CT scanning of the adrenal gland was normal. She refused to undergo adrenal vein sampling. At the last visit in July 2011, blood pressure was 130/85 mm Hg under candesartan 16 mg, nifedipine 60 mg, and spironolactone 25 mg, and serum potassium level was normal (4.0 meq/L, without supplements). Interestingly, the corrected QT was slightly prolonged (456 ms), despite the ECG being performed after correction of potassium levels.

A daughter was born in 2006 and referred to hospital for polyuria and polydipsia at 2 years of age. She was found hypertensive, hypokalemic, and severely hyperaldosteronemic (Table 2). She also displayed hypotonic urine (gravity: 1002 mg/dL) and hypercalciciuria (urinary Ca2+/creatinine ratio: 0.92). 18-Hydroxycortisol level was in the range of PA (serum potassium: 2.9 under potassium supplementation). Other clinical and hormonal parameters are summarized in Table 2. Long-PCR test for FH-I/GRA was negative, and hybrid steroid levels were within the normal range (Table 2) for patients with sporadic PA.16 CT scanning of the adrenal gland was normal. She refused to undergo adrenal vein sampling. At the last visit in July 2011, blood pressure was 130/85 mm Hg under candesartan 16 mg, nifedipine 60 mg, and spironolactone 25 mg, and serum potassium level was normal (4.0 meq/L, without supplements). Interestingly, the corrected QT was slightly prolonged (456 ms), despite the ECG being performed after correction of potassium levels.

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K+ (Figure S1B and S1C). The depolarized membrane voltage and the unchanged current size during replacement of Na+ by K+ indicate that the mutated channel complex is no longer K+ selective but similarly permeable for Na+ and K+. Interestingly, Ba2+ had almost no effect on the mutant channel complex; the current was only reduced by 5%. However, when the Na+ was replaced by the larger cation NMDG+, the whole cell inward current was almost abolished and the membrane hyperpolarized in cells expressing the mutant channel complex. Thus, the electrophysiological data clearly demonstrate a loss of K+ selectivity when the wild-type KCNJ5 subunit is replaced by KCNJ5^G151E. Instead of a K+ selective pore, the mutant channel allows Na+ and K+ to pass through the membrane. Taken together, the properties of KCNJ5^G151E are very similar to those reported recently for other mutations of KCNJ5.12

**Discussion**

In this study we investigated the presence of KCNJ5 mutations in 46 patients from 21 families considered previously as being affected by FH-II. Until 2011, only 1 form of FH had a recognized genetic cause, FH-I/GRA, in which a chimeric gene arising from a recombination between CYPIIB1 and CYP11B2 is responsible for aldosterone production under corticotropin control.5 Families with 2 PA affected members but without the hybrid gene were considered as affected by FH-II.6-10,11,16 In 2008, Geller et al7 reported an American family with a peculiar phenotype characterized by extremely severe PA and hypertension, resistant to multiple drug therapy, thus requiring bilateral adrenalectomy. This condition, named FH-III,22 was also characterized by a massive production of the hybrid steroids 18-hydroxycortisol and 18-oxocortisol and a paradoxical increase of aldosterone and blood pressure levels after DST.7 Recently, Choi et al,12 demonstrated that a germline T158A mutation in KCNJ5 was responsible for the disease and that another 2 somatic mutations in the same gene (G151R and L168R) were frequent in sporadic APA.12

In this study we demonstrate the presence of a newly described germline mutation in KCNJ5, responsible for FH. Interestingly, the phenotype of the 2 affected members was much milder compared with the American family. In particular, blood pressure levels and hypokalemia were easily corrected with medical therapy, which included MR antagonists and amiloride. The adrenals appeared normal by CT scanning, and hybrid steroids were produced at a much lower rate, in the range of other patients with sporadic PA. Furthermore, blood pressure and aldosterone levels were not paradoxically increased during DST. Recently, a KCNJ5 mutation (G387R) has been associated to long QT syndrome.23 This mutation was associated with a decreased activity of the channel because of a reduced expression of the protein at the plasma membrane. Interestingly, our index case with G151E mutation also displayed a mildly prolonged QT.

In KCNJ5, G151 lies in the selectivity filter at a position conserved among most K+ channels.12 In vitro studies of this mutation demonstrated a striking loss of K+ selectivity. The electrophysiological consequences of the G151E mutation investigated in the present study are thereby very much reminiscent of the mutations described recently by Choi et al.12 The G151E mutant channel was similarly permeable for Na+ and K+, resulting in depolarization of the plasma membrane and a continuous Na+ influx. In adrenocortical cells, such a depolarization would activate voltage-gated Ca2+ channels and disturb the K+ sensory function of glomerulosa cells. Even more dramatic are probably the cellular consequences of the Na+ influx: the rise of the cytosolic Na+ concentration leads to cell swelling and impair elimination of Ca2+ via Na+/Ca2+ exchangers.24 At very much depolarized membrane voltages and high intracellular Na+, the driving forces could be inverted and Na+/Ca2+ exchangers might even work in reverse mode, that is, import of Ca2+ instead of extrusion. Therefore, the novel KCNJ5 mutation G151E is potentially capable of modifying the cell biology of adrenocortical cells profoundly by disturbing membrane voltage and Ca2+ homeostasis explaining autonomous hormone production and disturbed cell cycle control.

The phenotypic heterogeneity in FH-III recalls that observed for FH-I/GRA, where the same genetic defect results in markedly different phenotypes between affected families5,16,25 and also within the same family.26 In line with this observation is the phenotype of the patient from Torino who carried a somatic mutation in the APA that correspond with the mutation (T158A) reported as being responsible for the severe phenotype in the American family. This patient displayed a phenotype indistinguishable from a patient with sporadic APA displaying hypertension grades 1 to 2. The observation in this study that 3 patients with apparent FH, treated by unilateral adrenalectomy, had KCNJ5 mutations present only in the APA raises questions about the classification of these 2 families as affected by FH and, therefore, diagnosis of FH in families with only 2 PA affected members should be performed with some caution. Two situations are theoretically possible: first, the 6 subjects of the 3 families were in fact affected by sporadic PA, and the KCNJ5 mutation was somatic and thus present only in the APA; this could be possible because the relatively high frequency of PA in the hypertensive population could result in a casual association of PA in 2 subjects of the same family; second, >1 genetic alteration is necessary to obtain a PA phenotype, and the 3 families display a genetic background predisposing to PA that developed into an APA only in patients carrying the KCNJ5 mutations and in BAH in the other subjects possibly affected by another genetic alteration. The difficulty in studying adrenal samples from bilateral forms of FH makes this conundrum unresolved at present.

**Perspectives**

The genetic cause of FH type III has been demonstrated recently to be attributed to a mutation on KCNJ5, contributing to the understanding of the pathophysiology of genetic and sporadic hyperaldosteronism and possibly to hypertension in general. We have demonstrated a new mutation in the gene encoding the Kir 3.4 potassium channel, causing an in vitro phenotype similar to that described by Choi et al12 but with a much milder clinical phenotype. Therefore, other patients with KCNJ5 mutations may have been incorrectly classified as affected by FH-II, and, thus, it would be interesting to
investigate the presence of mutations in \textit{KCNJ5} in other series of FH-II families. Future research will better define the clinical spectrum of alterations determined by \textit{KCNJ5} mutations and the role of this channel in the pathophysiology of hypertension and PA.

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**Disclosures**

None.

**References**


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KCNJ5 Mutations in European Families with Non-Glucocorticoid Remediable Familial Hyperaldosteronism

Paolo Mulatero*1, Philipp Tauber*2, Maria Christina Zennaro*3,4,5, Silvia Monticone1, Katharina Lang6, Felix Beuschlein7, Evelyn Fischer7, Davide Tizzani1, Anna Pallauf2, Andrea Viola1, Laurence Amar3,4,5, Tracy Ann Williams1, Tim M. Strom8, Elisabeth Graf8, Sascha Bandulik2, David Penton2, Pierre François Plouin3,4,5, Richard Warth2, Bruno Allolio6, Xavier Jeunemaitre3,4,5, Franco Veglio1, Martin Reincke7.

*These authors contributed equally to the study
1 Division of Internal Medicine and Hypertension, University of Torino, Torino, 10126 Italy
2 Medical Cell Biology, University Regensburg, Regensburg, Germany
3 INSERM, U970, Paris Cardiovascular Research Center, Paris, France
4 University Paris Descartes, Paris, France
5 Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France
6 Department of Medicine I, Endocrine and Diabetes Unit, University Hospital Würzburg, 97080 Würzburg, Germany
7 Medizinische Klinik Campus Innenstadt, Klinikum der LMU, Ziemssenstr. 1, D-80336 Munich, Germany
8 Institute of Human Genetics, Helmholtz Zentrum München, Ingolstädter Landstr. 1, 85764 Neuherberg

Corresponding author:
Paolo Mulatero, Division of Internal Medicine and Hypertension, AOU San Giovanni Battista, Via Genova 3, 10126, Torino, Italy; e-mail: paolo.mulatero@libero.it; fax:-39-011-6602707 ph:-39-011-6336959
### Expanded Methods

#### Diagnosis of PA and FH and exclusion of FH-I/GRA

**Torino**

Diagnosis of PA was performed as described in detail elsewhere (1). Briefly, patients were screened using the aldosterone (PAC)/plasma renin activity (PRA) ratio (ARR) and PA was confirmed using the intravenous saline load test. All patients with confirmed PA underwent CT scanning and adrenal vein sampling (AVS). AVS was considered successful if the adrenal vein/inferior vena cava cortisol gradient was at least 3 (2) (or at least 2 before 2008); lateralization was considered when the aldosterone/cortisol ratio (A/C) from one adrenal was at least 4 times that of the other adrenal gland. A definitive diagnosis of APA was made as defined elsewhere (1). Diagnosis of FH-I/GRA was excluded by long-range PCR as described previously (3). Diagnosis of FH-II was made when at least two members of the same family displayed confirmed PA and a negative long-range PCR test for FH-I/GRA as described in detail in the PATOGEN study (4). Hormone measurements were performed as described previously (1). A dexamethasone suppression test (DST) was performed only in patients carrying mutations in KCNJ5, as described previously (5).

**Paris**

Diagnosis of PA was performed as described in detail elsewhere (6). Patients with hypokalemic hypertension or resistant hypertension were screened for PA that was considered present if ARR was at least 64 pmol/mU (107 pmol/ng) in two separate measurements and aldosterone concentration exceeded 500 pmol/l (18 ng/dl) or urinary aldosterone excretion exceeded 63 nmol/day (23 mcg/day). Two patients underwent a saline infusion test and five patients underwent an adrenal venous sampling. Thin-slice CT scanning and AVS for subtype differentiation were performed and interpreted as described before (6,7). Renin was measured as direct active renin by a direct radioimmunoassay (ERIA renin active Pasteur CT, reference 79970, Paris, France). Plasma aldosterone was measured by radioimmunoassay.

**Munich and Würzburg**

The diagnosis of PA, was established as published previously (8). In brief, screening was performed by measurement of the aldosterone to renin ratio (ARR, cut-off 12.1 ng/L), followed by confirmatory testing using saline infusion suppression. Medication known to influence the renin aldosterone ratio was stopped whenever possible or adjusted according current guidelines. The diagnosis of unilateral aldosterone excess, mainly aldosterone producing adenoma, was based on adrenal vein sampling (AVS). In short, blood was drawn consecutively from both adrenal veins without ACTH stimulation. A selectivity index of $\geq 2.0$ of the cortisol concentrations within the adrenal vein compared to a simultaneously drawn peripheral sample was applied. Unilateral disease was assumed if the lateralisation index of the aldosterone to cortisol ratio was $\geq 4.0$ of the dominant versus the non-dominant side. In patients without AVS (<5% since 2004) unilateral disease was assumed if computed tomography or magnetic resonance imaging of the adrenal glands demonstrated a unilateral nodular enlargement and a contralateral normal appearing adrenal gland. Plasma aldosterone levels were measured using the radioimmunoassay ”Aldosterone Coat-a-Count” (Siemens Germany), and plasma direct renin levels by radioimmunoassay Renin III generation (Cisbio, Codolet, France).

### Sequencing

**DNA and RNA isolation and RT-PCR**

Genomic DNA was extracted from 46 patients with FH (27 from Torino, 10 from Paris, 5 from Munich, 4 from Würzburg), and from 79 unaffected relatives (60 from Torino, 2 from Würzburg, 13 from Munich and 4 from Paris). DNA or RNA was extracted from a total of 4 APA (1 from Torino, 1 from Paris and 2 from Würzburg). Tumor DNA was extracted using QIAamp DNA midi kit (Qiagen); DNA from peripheral blood leukocytes was prepared using salt-extraction or using the Blood & Cell Culture DNA Kit (Qiagen). Total RNA was isolated from frozen tissue using Trizol
(Invitrogen, Carlsbad, Ca, USA) and then cleaned-up on silica columns using RNeasy Micro Kit (Qiagen). The integrity and quality of the RNA were systematically checked using an Agilent 2100 bioanalyzer with the RNA6000 Nano Assay (Agilent Technologies). After DNaseI treatment (Invitrogen), 500 ng of total RNA were retro-transcribed using Superscript II RT (Invitrogen) and random hexamers (Promega, Madison, WI, USA).

**KCNJ5 sequencing**

Coding sequence of genomic DNA was investigated by exome sequencing. The KCNJ5 coding sequence spanning amino acids 122 to 199 was amplified and sequenced as previously reported (9). PCR was performed on 100 ng of DNA (4 µl of RT product) in a final volume of 25 µl containing 0.75 mM MgCl2 (without MgCl2 for cDNA), 400 nM of each primer, 200 µM deoxynucleotide triphosphate and 1.25 U Platinum Taq DNA Polymerase (Invitrogen). Cycling conditions were as previously described with an annealing temperature of 58°C (56°C for cDNA). Direct sequencing of PCR products was performed using the ABI Prism Big Dye Terminator® v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). Sequencing primers were as previously described (9).

**Functional Study of the G151E Mutation**

For functional expression in mammalian cells, specific cDNAs of human KCNJ3, KCNJ5 and mutant KCNJ5(151E) were purchased from Invitrogen/Geneart and subcloned into the pIRES-CD8 expression vector (10). For functional studies in HEK293 cells, wild type human KCNJ3 was cotransfected with wildtype KCNJ5 or with mutated KCNJ5(151E) using Lipofectamin. Patch clamp recordings were performed using an EPC-10 amplifier without leak subtraction (HEKA, Germany). The following solutions were used: control solution (10 mM HEPES pH 7.4, 140 mM NaCl, 5 mM KCl, 1.8 mM MgCl2, 1.8 mM CaCl2), 15 mM K+ solution (10 mM HEPES pH 7.4, 130 mM NaCl, 15 mM KCl, 1.8 mM MgCl2, 1.8 mM CaCl2), 50 mM K+ solution (10 mM HEPES pH 7.4, 95 mM NaCl, 50 mM KCl, 1.8 mM MgCl2, 1.8 mM CaCl2), Na+-free solution (10 mM HEPES pH 7.4, 5 mM KCl, 1.8 mM MgCl2, 1.8 mM CaCl2, 140 mM N-methyl-D-glucamine chloride (NMDG+)), pipette solution (5 mM HEPES pH 7.4, 140 mM KCl, 4 mM MgCl2, 1 mM CaCl2, 1 mM EGTA).

**References**


Supplemental Table S1. Clinical and hormonal parameters of patients with somatic KCNJ5 mutations after adrenalectomy.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TO Fam 7 Index Case</th>
<th>WU Fam</th>
<th>PA Fam</th>
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<tbody>
<tr>
<td>SBP/DBP (mmHg)</td>
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<td>135/90</td>
<td>128/90</td>
</tr>
<tr>
<td>sK+ (mEq L⁻¹)</td>
<td>4.4</td>
<td>4.4</td>
<td>4.4</td>
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<tr>
<td>upright sAldosterone (ng dL⁻¹)</td>
<td>7.5</td>
<td>3.5</td>
<td>9.6</td>
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<tr>
<td>upright PRA (ng mL⁻¹ h⁻¹)</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>DAR (pg mL⁻¹)</td>
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<td>19</td>
</tr>
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<td>single nodule 20 mm with normal surrounding cortex</td>
<td>21 mm nodule with micronodular hyperplasia</td>
<td>n.a.</td>
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<tr>
<td>number of drugs</td>
<td>1/1</td>
<td>3/0</td>
<td>1/0</td>
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</table>

SBP/DBP= systolic/diastolic blood pressure; sK+= serum potassium; PRA= plasma renin activity; DAR= direct active renin; n.a.= not available; to convert aldosterone to nmol/L multiply by 0.0277; to convert PRA to ng L⁻¹s⁻¹ multiply by 0.2778, to convert renin to pmol/L multiply by 0.0237.
Supplemental figure S1.

Legend to supplemental figure S1. Functional effects of the G151E mutation in HEK cell co-transfected with KCNJ5/KCNJ3 or KCNJ5G151E/KCNJ3. (A) Typical patch clamp current traces of a KCNJ5/KCNJ3-tranfected cell. A strong inward current was observed at 50 mM extracellular K⁺ that was progressively decreased when extracellular K⁺ was reduced. Replacement of Na⁺ by the large cation NMDG⁺ had almost no effect on the whole cell current. (B) Typical patch clamp current traces of a KCNJ5G151E/KCNJ3-tranfected cell. The whole cell current was slightly inwardly rectifying and insensitive to reduction of K⁺ from 50 to 5 mM. However, replacement of Na⁺ by NMDG strongly reduced the whole cell current. (C) Summary of similar experiments. Please note that the I/V relationship of KCNJ5/KCNJ3 expressing cells (n=8) is strongly influenced by extracellular K⁺, but almost not modified, when Na⁺ is replaced by NMDG⁺. In KCNJ5G151E/KCNJ3 expressing cells (n=9), modification of extracellular K⁺ has almost no effect. Na⁺ replacement by
NMDG\textsuperscript{+}, however, strongly reduces the current shifts the voltage at current = 0 to more hyperpolarized values.