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Complementary somatic mutations of *KCNJ5*, *ATP1A1* and *ATP2B3* in sporadic aldosterone producing adrenal adenomas

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Dear Editor,

Primary aldosteronism (PA) is the most common form of secondary hypertension, accounting for 8-13% among hypertension patients (Mulatero, et al. 2013). It is characterized by constitutive production of aldosterone by the adrenal cortex. Among the subtypes of PA, aldosterone producing adenomas (APAs), also known as Conn tumors, are characterized by tumors in the adrenal cortex and account for 30-40% of the cases. The two most important physiological stimuli of aldosterone secretion are angiotensin II and serum potassium. Decrease in blood volume activates the renin-angiotensin system in which angiotensin II signals via the angiotensin receptor. The K^+ concentration across the membrane sets the resting membrane potential. Hyperkalemia causes depolarization of the membrane and generates an action potential to open a voltage gated Ca^{2+} channel. In both cases, enhanced intracellular Ca^{2+} provides the normal signal for aldosterone production. In APAs, autonomous production of aldosterone is found independently of angiotensin II.

Recently, next generation sequencing has revealed novel genes frequently mutated in APAs: *KCNJ5, ATP1A1* and *ATP2B3* (Beuschlein, et al. 2013; Choi, et al. 2011; Mulatero et al. 2013; Taguchi, et al. 2012). In these pivotal studies, mutations in *KCNJ5*, encoding an inwardly rectifying K^+ channel, were identified in about 30-45% of patients. The K^+ channel encoded by *KCNJ5* exists both as homo-tetramer and as a hetero-tetramer with another potassium channel encoded by *KCNJ3*. The latter has been found more active than homo-tetramers (Choi et al. 2011). More recently, mutations in *ATP1A1* (encode a Na+/K+ pump ATPase α subunit) and *ATP2B3* (plasma membrane Ca^{2+}ATPase) were reported, each of which appears in about 6% and 2% of the tumors, respectively (Beuschlein et al. 2013). In the present study, we investigated *KCNJ5, KCNJ3, ATP1A1* and *ATP2B3* for mutations in a series of 35 consecutive patients with sporadic APAs from Norway, Sweden and Germany (protocols and primers available on request).

We found frequent somatic mutations in *KCNJ5, ATP1A1* and *ATP2B3.* No mutations were identified in *KCNJ3* which is in agreement with previous reports (Beuschlein et al. 2013; Choi et al. 2011; Taguchi et al. 2012).

Regarding *KCNJ5* (NM_000890.3), 11 (31%) missense mutations were identified. Seven mutations were at c.451G>A (p.Gly151Arg), one at c.451G>C (p.Gly151Arg) and three at c.503T>G (p.Leu168Arg) (Fig. 1a, 1b & 1c, respectively). The overall mutation frequency was in agreement with previous reports (Choi et al. 2011; Taguchi et al. 2012). Notably, the somatic mutations G151R and L168R are situated on the highly conserved Glycine-Tyrosine-Glycine (GYG) motif of the selective filter and the second transmembrane (TM) domain of KCNJ5, respectively (Heginbotham, et al. 1992). The GYG motif in the extracellular loop of all four subunits of the *KCNJ5* channel forms the narrowest part of the pore. Both mutations abolish the highly conserved region of the GYG motif. In *in vitro* studies, it appears that all mutations potentially lead to a loss of ion selectivity of the channel protein (Choi et al. 2011). Furthermore, reduction of inward K^+ current results in enhanced depolarization of the adrenal cells which leads to activation of voltage gated Ca^{2+} channel. An increase in intracellular Ca^{2+} is associated with higher aldosterone production.

Regarding *ATP1A1*, two missense variants (6%) were identified at c.311T>G (p.Leu104Arg) (Fig. 1d). Concerning *ATP2B3*, three inframe deletions (9%) were found, two of c.1272_1277delGCTGGT (p.Leu425-Val426del) and one of c.1281_1286delGGCTGT (p.Arg428-Val429del) (Fig. 1e & 1f). The overall mutation frequencies were slightly higher than in one previous report (Beuschlein et al. 2013) which may be due to small sample size. Of note,we identified the novel mutation c.1281_1286delGGCTGT in *ATP2B3*.

The protein encoded by both genes *ATP1A1* and *ATP2B3* exchanges K^+ and Ca^{2+} ions, respetively, by hydrolysis of one ATP (Di Leva, et al. 2008; Kaplan 2002). On the crystal structure of *ATP1A1*, the mutant L104R is located in the transmembrane α helix M1, which has been suggested to interact and cooperate in K^+ ion binding and gating by interaction with Glu334 (Morth, et al. 2007). It has been found that angiotensin II inhibits the Na^+/K^+ pump activity for aldosterone production in glomerulosa cells

(Hajnoczky, et al. 1992). Since Ca^{2+} ion pumps are highly conserved, we used sarcoplasmic reticulum type Ca^{2+} ATPase (SERCA) to project the mutations. The deletions 425Ala_426Val and 428Ala_429Val corresponds to 303Ala_304Val and 306Ala_307Ile (Fig. 1g). The PEGLP motif after Ile307 is a key motif for ion gating and is highly conserved among the P type pumps (Di Leva et al. 2008). Mutations potentially lead to the distortion of this Ca^{2+} binding region. Notably, in both ATPase genes, the mutation abolishes Glu334 and Glu309 in *ATP1A1* and *ATP2B3* that are crucially important for ion gating. Functional *ex vivo* studies of the role of the loss of function mutations in the ATPase genes (Beuschlein et al. 2013) showed substantially higher levels of depolarization in the mutated samples.

In this study, the expression of *KCNJ5* at the mRNA level was found to be significantly lower in mutated samples (P=0.02) (Fig. 1h). This finding is in disagreement with previous results (Boulkroun, et al. 2013; Taguchi et al. 2012). The reason for this discrepancy might be the rather small sample size. In contrast to *KCNJ5*, the mRNA expression levels of *ATP1A1* and *ATP2B3* were not affected by mutational status (Fig. 1i & 1j, respectively). This is in agreement with previous results (Beuschlein et al. 2013).

Clinical characteristics of the patients are shown in Table 1. In contrast to patients with *KCNJ5* mutations, ATPase mutated APAs were predominantly found in males (Table 1). There was no statistically significant difference concerning the age of patients having APAs with different mutations (Fig. 1k). While the tumor size of APAs with somatic *KCNJ5* mutations was almost twice the size of APAs with either somatic *ATP1A1* and *ATP2B3* mutations, this difference was not statistically significant (Fig. 1l). No conclusions could be drawn from the preoperative aldosterone levels (Fig. 1m).

In conclusion, somatic mutations found in *KCNJ5, ATP1A1* and *ATP2B3* appear to be driving forces for a higher aldosterone production and proliferations of glomerulosa cells. All mutations found in this study were complementary to each other (Fig. 1n) indicating that multiple genes may contribute independently to the formation of APAs.

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Declaration of interest

The authors declare that they have no conflict of interest.

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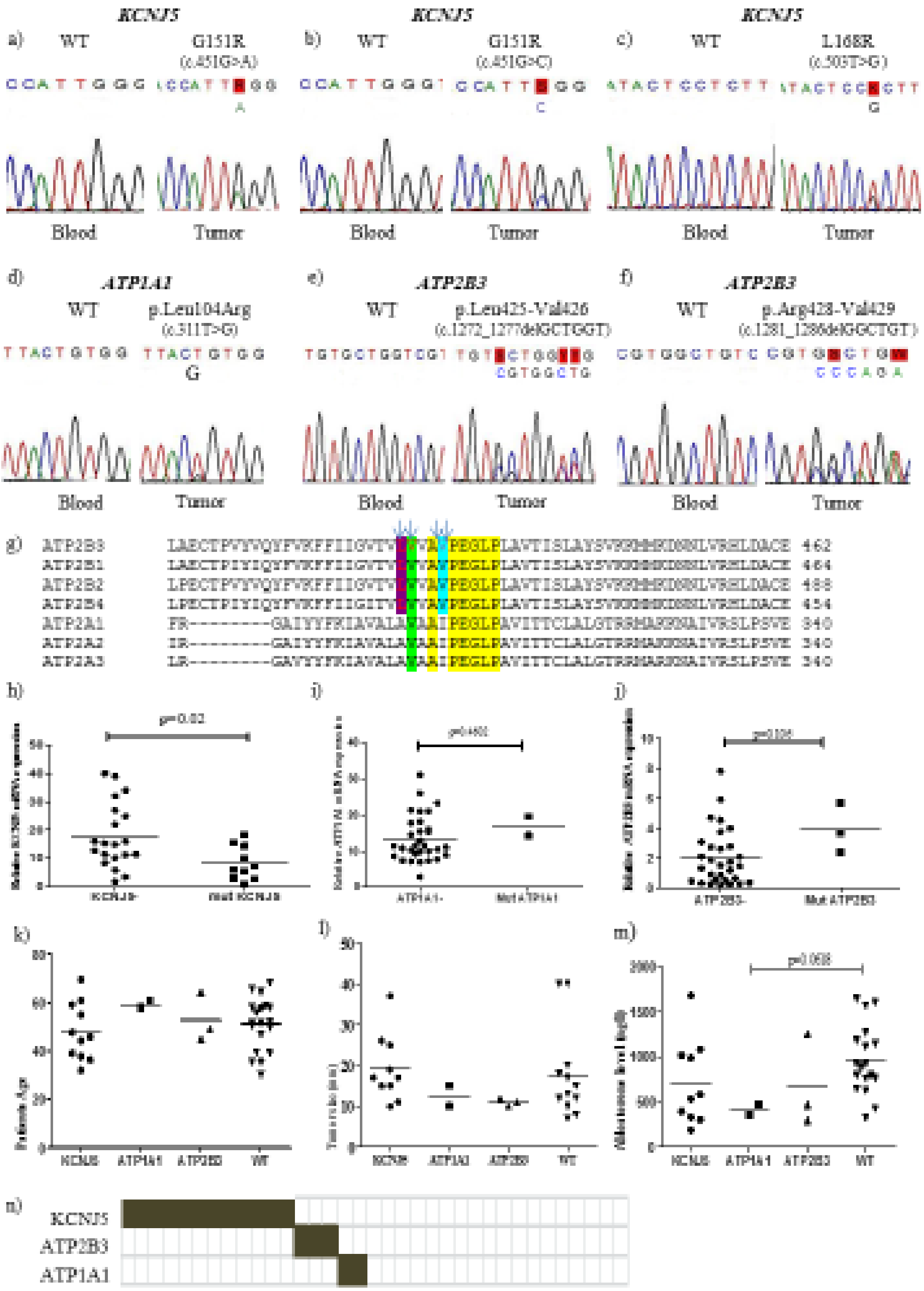
Figure 1.



a)
KCNJ5
WT
G151R
(c.451G>A)
Blood
Tumor
b)
KCNJ5
WT
G151R
(c.451G>C)
Blood
Tumor
c)
KCNJ5
WT
L168R
(c.503T>G)
Blood
Tumor
d)
ATP1A1
WT
p.Leu104Arg
(c.311T>G)
Blood
Tumor
e)
ATP2B3
WT
p.Leu425-Val426
(c.1272_1277delGCTGGT)
Blood
Tumor
f)
ATP2B3
WT
p.Arg428-Val429
(c.1281_1286delGGCTGT)
Blood
Tumor
g)
ATP2B3
ATP2B1
ATP2B2
ATP2B4
ATP2A1
ATP2A2
ATP2A3
h)
i)
j)
k)
l)
m)
n)
KCNJ5
ATP2B3
ATP1A1

Table 1: Clinical characteristic of 16 APA patients with different mutations in *KCNJ5*, *ATP1A1* and *ATP2B3*

sample	age (years)	sex	preop aldo (ng/l)	size (mm)	gene	cDNA bp
L1	39.1	M	580	7	*KCNJ5*	c.451G>A
L15	49.1	F	290	10	*ATP2B3*	c.1281_1286delGGCTGT
L37	58.0	M	470	10	*ATP1A1*	c.311T>G
L58	45.9	F	530	10	*KCNJ5*	c.451G>C
L70	32.1	F	980	25	*KCNJ5*	c.503T>G
B1	64.3	M	1246	11	*ATP2B3*	c.1272_1277delGCTGGT
B2	37.7	F	1675	17	*KCNJ5*	c.503T>G
B9	36.4	F	1013	37	*KCNJ5*	c.451G>A
B17	47.7	M	1078	17	*KCNJ5*	c.451G>A
G1	54.9	M	300	26	*KCNJ5*	c.451G>A
G2	60.5	M	350	15	*ATP1A1*	c.311T>G
G3	69.4	F	329	19	*KCNJ5*	c.503T>G
G4	60.9	M	184	15	*KCNJ5*	c.451G>A
G6	59.1	F	NA	11	*KCNJ5*	c.451G>A
L131	44.2	F	390	15	*KCNJ5*	c.451G>A
L141	44.8	M	460	12	*ATP2B3*	c.1272_1277delGCTGGT

M=Male
F=Female
NA=Not available
mm=millimeters
ng= Nanogram
l= liter

Sequences of blood DNA showing no mutation (WT) and mutated tumor DNA showing the following somatic missense mutations c.451G>A (**a**), c.451G>C (**b)** and c.503T>G (**c**). Normal blood and mutated tumor DNA sequences regarding *ATP1A1* (c.311T>G) (**d**), c.1272_1277delGCTGGT *ATP2B3* (**e**) and c.1281_1286delGGCTGT (**f**), respectively).

Alignment of plasma membrane $Ca2^{+}$ ATPase pumps and sarcoplasmic reticulum type $Ca2^{+}$ATPases (**g**). Colored region are conserved among them. The arrow indicates the deleted residues in our cases. The PEGLP motif is conserved among all p-type pump. It is a key factor for ion gating.

mRNA expression of *KCNJ5* in APAs with mutation (Mut KCNJ5) and without *KCNJ5* mutation (KCN5-) (**h**). The mRNA levels of mutated *KCNJ5* were significantly lower (p=0.02). Expression of *ATP1A1* mRNA of APAs with (Mut ATP1A1) and without mutation (*ATP1A1*-) (**i**). Expression of *ATP2B3* mRNA in APAs with (Mut ATP2B3) and without mutation (ATP2B3-) (**j**).

Age of patients with APAs with regard to the somatic mutation (*KCNJ5*, *ATP1A1* and *ATP2B3*)(**k**). Diameter of APAs with regard to the somatic mutation (**l**). Comparison of aldosterone levels of the patients with APAs with regard to the somatic mutation (*KCNJ5*, *ATP2B3* and *ATP1A1*) (**m**). Lines show the mean value of each group.

Complementary mutations of *KCNJ5*, *ATP2B3* and *ATP1A*. Mutation frequencies of 31% for *KCNJ5*, 9% for *ATP2B3* and 6% for *ATP1A1* were observed in our cohort (**n**).