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

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 Ca²⁺ channel. In both cases, enhanced intracellular Ca²⁺ 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 (Choi et al. 2011, Taguchi et al. 2012, Beuschlein et al. 2013, Mulatero et al. 2013). 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 Ca²⁺ ATPase) have been reported, each of which appears in about 6 and 2% of the tumors respectively (Beuschlein et al. 2013). In this 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 (Choi et al. 2011, Taguchi et al. 2012, Beuschlein et al. 2013).

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, b and c 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 cell membranes, which leads to activation of voltage-gated Ca²⁺ channels. An increase in intracellular Ca²⁺ 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_1277delGCTGTT (p.Leu425-Val426del) and one of c.1281_1286delGGCTGT (p.Arg428-Val429del) (Fig. 1e and f). 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.
Complementary mutations in Conn tumors

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Homologue</th>
<th>Tumor</th>
<th>Blood</th>
</tr>
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<tbody>
<tr>
<td>KCNJ5</td>
<td>G151R (c.451G&gt;A)</td>
<td>WT</td>
<td>Tumor</td>
<td>Blood</td>
</tr>
<tr>
<td>KCNJ5</td>
<td>G151R (c.451G&gt;C)</td>
<td>WT</td>
<td>Tumor</td>
<td>Blood</td>
</tr>
<tr>
<td>KCNJ5</td>
<td>L168R (c.503T&gt;G)</td>
<td>WT</td>
<td>Tumor</td>
<td>Blood</td>
</tr>
</tbody>
</table>

- ATP2B3: p.Leu425-Val426 (c.1272_1277delGCTGGT)
- ATP2B3: p.Arg428-Val429 (c.1281_1286delGGCTGT)
- ATP2B3: p.Leu104Arg (c.311T>G)

**Gene Expression**

- **KCNJ5**: Relative mRNA expression
  - WT: 0, mut: 1
  - P = 0.02

- **ATP1A1**: Relative mRNA expression
  - WT: 0, Mut: 1
  - P = 0.4802

- **ATP2B3**: Relative mRNA expression
  - WT: 0, Mut: 1
  - P = 0.106

**Clinical Parameters**

- Patients age
  - KCNJ5: 0, ATP1A1: 50, ATP2B3: 100, WT: 150
  - P = 0.0618

- Tumor size (mm)
  - KCNJ5: 0, ATP1A1: 10, ATP2B3: 20, WT: 30
  - P = 0.02

- Aldosterone level (ng/l)
  - KCNJ5: 0, ATP1A1: 500, ATP2B3: 1000, WT: 1500
  - P = 0.02
The protein encoded by both genes *ATP1A1* and *ATP2B3* exchanges K\(^+\) and Ca\(^{2+}\) ions, respectively, by hydrolysis of one ATP (Kaplan 2002, Di Leva et al. 2008). On the crystal structure of *ATP1A1*, the mutant L104R is located in the TM \(\alpha\) 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). As 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 mutant cells.

In this study, the expression of *KCNJ5* at the mRNA level was found to be significantly lower in mutated samples \((\text{P} = 0.02)\) (Fig. 1h). This finding is in disagreement with previous results (Taguchi et al. 2012, Boulkroun et al. 2013). 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 and j 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).

Although the tumor size of APAs with somatic *KCNJ5* mutations was almost twice the size of APAs with either

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Preoperative aldosterone (ng/l)</th>
<th>Size (mm)</th>
<th>Gene</th>
<th>cDNA bp</th>
</tr>
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<tbody>
<tr>
<td>L1</td>
<td>39.1</td>
<td>M</td>
<td>580</td>
<td>7</td>
<td>KCNJ5</td>
<td>c.451G&gt;A</td>
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<tr>
<td>L15</td>
<td>49.1</td>
<td>F</td>
<td>290</td>
<td>10</td>
<td>ATP2B3</td>
<td>c.1281_1286delGGCTGT</td>
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<tr>
<td>L37</td>
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<td>M</td>
<td>470</td>
<td>10</td>
<td>ATP1A1</td>
<td>c.311T&gt;G</td>
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<tr>
<td>L58</td>
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<td>F</td>
<td>530</td>
<td>25</td>
<td>KCNJ5</td>
<td>c.451G&gt;C</td>
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<tr>
<td>L70</td>
<td>32.1</td>
<td>F</td>
<td>980</td>
<td>11</td>
<td>ATP2B3</td>
<td>c.503T&gt;G</td>
</tr>
<tr>
<td>B1</td>
<td>64.3</td>
<td>M</td>
<td>1246</td>
<td>17</td>
<td>KCNJ5</td>
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<tr>
<td>B2</td>
<td>37.7</td>
<td>F</td>
<td>1675</td>
<td>37</td>
<td>KCNJ5</td>
<td>c.451G&gt;A</td>
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<tr>
<td>B9</td>
<td>36.4</td>
<td>F</td>
<td>1013</td>
<td>17</td>
<td>KCNJ5</td>
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<tr>
<td>B17</td>
<td>47.7</td>
<td>M</td>
<td>1078</td>
<td>26</td>
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<tr>
<td>G1</td>
<td>54.9</td>
<td>M</td>
<td>300</td>
<td>15</td>
<td>ATP1A1</td>
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</tr>
<tr>
<td>G2</td>
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<td>19</td>
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<tr>
<td>G3</td>
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<td>15</td>
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<td>c.451G&gt;A</td>
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<tr>
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<td>M</td>
<td>184</td>
<td>11</td>
<td>KCNJ5</td>
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</tr>
<tr>
<td>G6</td>
<td>59.1</td>
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<tr>
<td>L131</td>
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<tr>
<td>L141</td>
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<td>M</td>
<td>460</td>
<td>12</td>
<td>ATP2B3</td>
<td>c.1272_1277delGCTGGT</td>
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</tbody>
</table>

M, male; f, female; NA, not available.
somatic ATP1A1 and ATP2B3 mutations, this difference was not statistically significant (Fig. 1). 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 the 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
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