Activating Mutation of the Renal Epithelial Chloride Channel ClC-Kb Predisposing to Hypertension

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Abstract—The chloride channel ClC-Kb is expressed in the basolateral cell membrane of the distal nephron and participates in renal NaCl reabsorption. Loss-of-function mutations of ClC-Kb lead to classic Bartter syndrome, a rare salt-wasting disorder. Recently, we identified the ClC-KbT481S polymorphism, which confers a strong gain-of-function effect on the ClC-Kb chloride channel. The present study has been performed to explore the prevalence of the mutation and its functional significance in renal salt handling and blood pressure regulation. As evident from electrophysiological analysis with the 2-electrode voltage-clamp technique, heterologous expression of ClC-KbT481S in Xenopus oocytes gave rise to a current that was 7-fold larger than the current produced by wild-type ClC-Kb. The prevalence of the mutant allele was significantly higher in an African population from Ghana (22%) than in whites (12%). As tested in 1 white population, carriers of ClC-KbT481S were associated with significantly higher systolic (by ≈6.0 mm Hg) and diastolic (by ≈4.2 mm Hg) blood pressures and significantly higher prevalence (45% versus 25%) of hypertensive (≥140/90 mm Hg) blood pressure levels. Individuals carrying ClC-KbT481S had significantly higher plasma Na⁺ concentrations and significantly decreased glomerular filtration rate. In conclusion, the mutation ClC-KbT481S of the renal epithelial Cl⁻ channel ClC-Kb strongly activates ClC-Kb chloride channel function in vitro and may predispose to the development of essential hypertension in vivo. (Hypertension. 2004;43:1175-1181.)

Key Words: blood pressure ■ ethnic groups ■ genes ■ glomerular filtration rate ■ hypertension, genetic ■ ion transport ■ kidney

A mple evidence points to a role of renal tubular sodium chloride (NaCl) reabsorption in the development of hypertension. Increased renal tubular reabsorption, eg, in mineralocorticoid excess or in genetic disorders leading to enhanced renal tubular Na⁺ reabsorption, are well-known causes of hypertensive disease. Approximately 20% of filtered Na⁺ are reabsorbed in the thick ascending limb of the loop of Henle (TAL). Thus, deranged reabsorption of Na⁺ in this nephron segment could impact on blood pressure regulation. Reabsorption of Na⁺ in the TAL is accomplished by entry of Na⁺ across the apical membrane via the Na⁺-K⁺-2Cl⁻ cotransporter (NKCC2 or BSC1). The cotransported K⁺ recirculates into the lumen via the renal outer medullary K⁺ channel ROMK. Across the basolateral cell membrane, Na⁺ is extruded by the Na⁺/K⁺-ATPase and Cl⁻ exits via Cl⁻ channels composed of the pore-forming unit ClC-Kb and the β-subunit barttin. The recirculation of positively charged K⁺ via ROMK across the apical cell membrane and the exit of negatively charged Cl⁻ via ClC-Kb/barttin across the basolateral cell membrane generate a lumen-positive transepithelial voltage-gradient that drives paracellular Ca²⁺ reabsorption. The significance of these transport pathways for blood pressure regulation is illustrated by the fact that inhibitors of NKCC2, ie, loop diuretics, are highly effective in antihypertensive treatment. Moreover, loss of function mutations of genes encoding NKCC2, ROMK, ClC-Kb, barttin, or ClC-Ka and ClC-Kb lead to renal salt wasting and hypotension.

Beyond their localization in the TAL of Henle’s loop, ClC-Kb and barttin are expressed in the macula densa and more distal segments of the nephron. Barttin, in addition, associates with the ClC-Kb homologue ClC-Ka in thin limbs of the loop of Henle and in the inner ear. Mutations of the ClC-Kb gene CLNKB lead to the classic Bartter syn-
drome, characterized by mild salt wasting, whereas a combined loss-of-function of CIC-Ka and CIC-Kb by mutations of the barttin gene BSND or digenic mutations in CLCNKA and CLCNKB cause severe renal salt wasting with antenatal onset, congenital deafness, and renal failure.

Most recently, voltage clamp experiments disclosed that a naturally occurring variation of the CLCNKB gene (1441 A→T; Acc. No. NM 000085.1), leading to the replacement of threonine by serine at the amino acid position 481 of the CIC-Kb protein (CIC-KbT481S), dramatically increases CIC-Kb chloride channel activity. In theory, enhanced activity of CIC-Kb should decrease cytosolic Cl− concentration, which, in turn, would enhance the driving force and transport rate of the luminal Na+, K+, 2Cl− cotransport, eventually stimulating transepithelial NaCl reabsorption. To the extent that enhanced reabsorption of Na+ in the TAL would lead to renal salt retention, carriers of the CIC-KbT481S mutation should be prone to increased blood pressure. The present study aimed to explore whether this mutation may impact on blood pressure regulation.

Methods

Functional Analysis of Mutated CIC-Kb

To verify the functional significance of the CIC-KbT481S mutation, *Xenopus laevis* oocytes were injected with cRNA-encoding wild-type barttin (5 ng/oocyte) together with 5 ng/oocyte of either wild-type CIC-KbV1 or CIC-KbT481S. After 3 days, the currents were determined in 2-electrode voltage-clamp experiments with a pulse protocol of 800 ms pulses from −140 mV to +40 mV in 20-mV increments from −60 mV. steady-state currents at the end of each voltage step were filtered at 10 Hz and recorded with MacLab digital to analog converter and software for data acquisition and analysis (AD Instruments, Castle Hill, Australia). The bath solution (ND96) contained (in mM) 96 NaCl, 2 KCl, 1.8 CaCl2, 1 MgCl2, and 5 HEPES, pH 7.4.

Volunteers

Students and employees of the University of Tübingen (whites) volunteered for blood pressure measurements and genetic analysis. No dietary recommendations were given and individuals undergoing antihypertensive treatment were not a priori excluded. Frequency distribution of the CIC-KbT481S mutation was further investigated in 3 additional groups recruited randomly from: (1) healthy blood donors; (2) patients with Bartter syndrome, 17,18 characterized by mild salt wasting, whereas a phenotype defined as statistically significant. For all calculations, the GraphPadPrism software package version 3.0 was used (GraphPad Software Inc, San Diego, Calif). All laboratory procedures were performed blind to case-control status.

Results

As shown in the Figure, in the presence of barttin, the current induced by CIC-KbT481S was significantly larger than the current induced by wild-type CIC-Kb.

The prevalence of the CIC-KbT481S mutation is given in Table 1. In 3 different white populations, a prevalence of approximately 20% for heterozygous CIC-KbT481S/CIC-Kb and of ≈ 2% of homozygous mutant individuals (CIC-KbT481S/CIC-KbT481S) was obtained. Allele and genotype frequencies were significantly different between the white and the Ghana population.
Enhanced conductance of ClC-Kb T481S as compared with wild-type ClC-Kb. Xenopus oocytes have been injected with mRNA encoding barttin and either ClC-Kb or ClC-Kb T481S. Three days later, Cl channel activity was estimated by dual-electrode voltage-clamp, ie, 800-ms pulses from −140 mV to 40 mV in 20-mV increments were applied and steady-state current at the end of each voltage step determined. A, Original tracings. B, Arithmetic means ±SEM (n = number of experiments) of currents in Xenopus oocytes expressing wild-type (wt) ClC-Kb/barttin or ClC-KbT481S/barttin. *Significant difference between currents in oocytes expressing wt ClC-Kb or ClC-Kb T481S.

The prevalence of carriers of ClC-Kb T481S was significantly higher in Africans as compared with any of the 3 white populations (Table 1).

Because the frequency distribution of homozygous carriers for the ClC-Kb T481S variant is low (Table 1), wild-type individuals were compared with the sum of subjects heterozygous or homozygous for the ClC-Kb T481S variant. Volunteers (students and employees) from the University of Tübingen did not show significant differences in age, gender, size, body weight, and body surface area between carriers of ClC-Kb T481S (ClC-Kb T481S/ClC-Kb and ClC-Kb T481S/ClC-Kb T481S) and “wild-type” individuals (ClC-Kb/ClC-Kb) (Table 2). In contrast, systolic and diastolic blood pressure values were significantly higher in carriers of ClC-Kb T481S than in carriers of ClC-Kb/ClC-Kb. A similar significant difference of blood pressure values was obtained at comparison of heterozygous ClC-Kb T481S/ClC-Kb with ClC-Kb/ClC-Kb. Three of the 6 individuals carrying ClC-Kb T481S/ClC-Kb T481S had hypertensive blood pressure values (≥140/90 mm Hg). One of them, however, was hypotensive (115/73 mm Hg). Because of the small sample size, the mean values from single blood pressure determinations were not significantly different between ClC-Kb T481S/ClC-Kb T481S and ClC-Kb/ClC-Kb.

As indicated in Table 2, the difference of blood pressure between carriers of ClC-Kb T481S and ClC-Kb/ClC-Kb wild-type individuals still holds true after correction for age. Male carriers of ClC-Kb T481S had again significantly higher blood pressure values than male ClC-Kb/ClC-Kb carriers. Female carriers of ClC-Kb T481S tended to have higher blood pressure values than female ClC-Kb/ClC-Kb carriers; however, the difference was not statistically significant.

After exclusion of blood pressure data from individuals undergoing antihypertensive treatment (n = 18), the systolic (P = 0.022) and diastolic (P = 0.015) blood pressure values were still significantly higher in carriers of ClC-Kb T481S than in ClC-Kb/ClC-Kb.

The incidence of hypertensive blood pressure values (≥140/90 mm Hg) was significantly (P = 0.01) higher in carriers of ClC-Kb T481S than in ClC-Kb/ClC-Kb (odds ratio 2.4). This result again holds true after exclusion of individuals undergoing antihypertensive treatment (P = 0.02, odds ratio 3.9). A similar significantly enhanced incidence of hypertensive blood pressure values was observed in male (P = 0.017; odds ratio 3.7) but not in female carriers of ClC-Kb T481S.

### TABLE 1. Allele and Genotype Frequency Distribution of the ClC-Kb T481S Mutation in Different Populations

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stud + Emp</td>
<td>Blood Donors</td>
<td>Volunteers</td>
<td>University</td>
</tr>
<tr>
<td></td>
<td>University</td>
<td>Tübingen</td>
<td>Bavaria</td>
<td>Tübingen</td>
</tr>
<tr>
<td></td>
<td>(n = 220)</td>
<td>(n = 463)</td>
<td>(n = 313)</td>
<td>(n = 131)</td>
</tr>
<tr>
<td>ClC-Kb/ClC-Kb</td>
<td>173 (78.6%)</td>
<td>355 (76.7%)</td>
<td>243 (77.6%)</td>
<td>78 (59.5%)</td>
</tr>
<tr>
<td>ClC-Kb T481S/ClC-Kb</td>
<td>41 (18.6%)</td>
<td>101 (21.8%)</td>
<td>61 (19.5%)</td>
<td>48 (36.6%)</td>
</tr>
<tr>
<td>ClC-Kb T481S/ClC-Kb T481S</td>
<td>6 (2.7%)</td>
<td>7 (1.5%)</td>
<td>9 (2.9%)</td>
<td>5 (3.8%)</td>
</tr>
<tr>
<td>A allele</td>
<td>387 (88.0%)</td>
<td>811 (87.6%)</td>
<td>483 (86.0%)</td>
<td>204 (77.8%)</td>
</tr>
<tr>
<td>T allele</td>
<td>53 (12.0%)</td>
<td>115 (12.4%)</td>
<td>79 (14.1%)</td>
<td>58 (22.1%)</td>
</tr>
</tbody>
</table>

For statistical analysis, the Fisher exact test and χ² test were used when appropriate (see Methods).

Stud indicates student; emp, employee.
To determine whether individuals with normal or elevated blood pressure might be covertly stratified, we analyzed the unlinked frequent genetic polymorphism C3435T of the MDR1 gene. For the total population, the frequency distribution of wild-type (CC), heterozygous (CT), and homozygous mutant (TT) individuals were 26%, 50%, and 24%, respectively, which are completely in line with the prevalence of this mutation in several large healthy German populations previously investigated.31 Average systolic and diastolic blood pressures were similar in CC3435 (129±18/80±11 mm Hg), C3435T (130±17/79±10 mm Hg), and 3435TT (132±18/80±11 mm Hg) carriers. There were no significant deviations from Hardy-Weinberg equilibrium for the C3435T polymorphism within heterozygous and homozygous mutant individuals of ClC-KbT481S and ClC-Kb wild-type subjects.

Single blood pressure measurements are biased by many extrinsic and intrinsic factors and thus may not reflect the true blood pressure state. To exclude most of the extrinsic factors, we performed repeated automatic blood pressure determinations in a subset of volunteers (n=67) during sleeping hours. As illustrated in Table 3, nocturnal blood pressure values were again significantly enhanced in individuals carrying the mutation.

Individuals carrying ClC-KbT481S had a significantly (P=0.019) higher plasma Na+ concentration and a significantly (P=0.05) smaller GFR than ClC-Kb/ClC-Kb. The fractional excretion of K+ was significantly (P=0.046) larger, whereas those of Na+, Ca2+, and phosphate were not significantly different in carriers of ClC-KbT481S as compared with ClC-Kb/ClC-Kb.

We further tested whether correction for renal salt excretion would abolish the differences in blood pressure values. To this end, a correlation between blood pressure values and renal salt excretion was calculated for the whole population and the individual blood pressure values corrected for the average influence of the individual salt excretion. After this correction, the systolic (P=0.001) and diastolic (P=0.004) blood pressure values were still significantly higher in carriers of ClC-KbT481S than in carriers of ClC-Kb/ClC-Kb.

**Discussion**

The present observations confirm the gain of function of ClC-KbT481S shown previously.25 More importantly, they disclose the significance of enhanced activity of ClC-Kb channels for hypertension. Even heterozygous individuals display significantly higher blood pressure, indicating that the parallel expression of ClC-KbT481S and wild-type ClC-Kb favors renal salt retention and subsequent increase of blood pressure.

The analysis of the mutation in a single population bears the risk that the population includes a subpopulation with distinct genetic background. In theory, an increased prevalence of the ClC-KbT481S mutation in this population may by chance be associated with another genetic alteration predisposing to hypertension. However, we have analyzed exclusively individuals with identical ethnic background (Middle European) and because the screening has been performed specifically to test for a single gain of function mutation, the
likelihood that the differences in blood pressure were caused by a different gene is rather modest. Moreover, to depict possible genetic inhomogeneity, we have screened for prevalence of a MDR1 gene polymorphism. As a result, the MDR1 gene polymorphism was not associated with increased blood pressure and, as expected, did not correlate with ClC-Kb T481S. Although the analysis of a single gene does not definitely rule out population stratification, the data do suggest that the population was not significantly stratified.

Because CIC-Kb is expressed exclusively in the kidney and the inner ear,10,11 the increase of blood pressure in individuals carrying the ClC-Kb T481S mutation must be the result of altered renal NaCl reabsorption. Enhanced CIC-Kb channel activity favors Cl– exit across the basolateral cell membrane leading to decrease of cytosolic Cl– activity and cell volume. Decrease of cytosolic Cl– activity should increase the driving force and cell shrinkage should stimulate the activity of the apical Na+–K+–2Cl– cotransporter, which were expected to increase transport rate after activation of the basolateral Cl– channels.33 Gain of function mutations of the renal epithelial Na+ channel ENaC have been shown before to underlie the severe hypertension in Liddle syndrome.2,34,35 The present observation reveals the second mutation in an epithelial ion channel causing increase of blood pressure. Unlike Liddle syndrome, the mutation described here is common, affecting 20% of a white population. Other monogenic hypertensive disorders are caused by deranged regulation of renal tubular NaCl transport, such as in Gordon syndrome,36 mutations of the mineralocorticoid receptor,17,38 mutations of 11-hydroxysteroiddehydrogenase39,40 and glucocorticoid remediation.

### TABLE 3. Summary of Nocturnal Blood Pressure Values, Blood Plasma Values of Electrolytes, Glomerular Filtration Rate, and Urinary Electrolyte Excretion in 67 Volunteers Who Were Carriers of ClC-Kb T481S or Homozygous Wild-Type Individuals for ClC-Kb

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ClC-Kb/ClC-Kb</th>
<th>ClC-Kb T481S/ClC-Kb and ClC-Kb T481S/ClC-Kb T481S</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>37 (16♀, 21♂)</td>
<td>30 (19♀, 11♂)</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>28.2±10</td>
<td>33.5±15</td>
<td>NS</td>
</tr>
<tr>
<td>Size, cm</td>
<td>174.4±11</td>
<td>172.7±10</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.4±17</td>
<td>66.6±12</td>
<td>NS</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.83±0.26</td>
<td>1.78±0.19</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP sleep</td>
<td>101.7±19</td>
<td>110.9±10</td>
<td>0.022</td>
</tr>
<tr>
<td>Diastolic BP sleep</td>
<td>61.6±7</td>
<td>64.9±9</td>
<td>NS</td>
</tr>
<tr>
<td>MAP sleep</td>
<td>75.0±9</td>
<td>80.2±9</td>
<td>0.028</td>
</tr>
<tr>
<td>Systolic BP 2 to 6 AM</td>
<td>101.9±10</td>
<td>109.6±11</td>
<td>0.004</td>
</tr>
<tr>
<td>Diastolic BP 2 to 6 AM</td>
<td>59.8±6</td>
<td>63.7±9</td>
<td>0.048</td>
</tr>
<tr>
<td>Systolic BP min</td>
<td>81.9±8</td>
<td>91.7±13</td>
<td>0.0005</td>
</tr>
<tr>
<td>Diastolic BP min</td>
<td>44.1±4</td>
<td>50.0±11</td>
<td>0.005</td>
</tr>
<tr>
<td>[Na+]p</td>
<td>140.6±1.7</td>
<td>141.7±1.9</td>
<td>0.019</td>
</tr>
<tr>
<td>[K+]p</td>
<td>3.9±0.3</td>
<td>3.9±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>[Ca+]p</td>
<td>2.44±0.07</td>
<td>2.42±0.08</td>
<td>NS</td>
</tr>
<tr>
<td>[Pi]p</td>
<td>3.4±0.5</td>
<td>3.3±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td>91.7±26</td>
<td>79.2±24</td>
<td>0.05</td>
</tr>
<tr>
<td>GFR/body surface area</td>
<td>49.7±9.7</td>
<td>43.9±12.3</td>
<td>0.0396</td>
</tr>
<tr>
<td>FE K+ %</td>
<td>1.0±0.44</td>
<td>1.07±0.45</td>
<td>NS</td>
</tr>
<tr>
<td>FE Na+ %</td>
<td>14.8±5</td>
<td>19.0±11</td>
<td>0.046</td>
</tr>
<tr>
<td>FE Ca2+ %</td>
<td>1.8±0.9</td>
<td>1.8±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>FE Pi %</td>
<td>21.8±6</td>
<td>25.6±10</td>
<td>NS</td>
</tr>
<tr>
<td>UVNa, mmol/24 h</td>
<td>169.1±74</td>
<td>142.2±54</td>
<td>NS</td>
</tr>
<tr>
<td>UVK, mmol/24 h</td>
<td>70.0±22</td>
<td>62.2±21</td>
<td>NS</td>
</tr>
<tr>
<td>UVPi, mmol/24 h</td>
<td>5.3±2</td>
<td>4.8±3</td>
<td>NS</td>
</tr>
<tr>
<td>UVK, mg/24 h</td>
<td>894±336</td>
<td>844±268</td>
<td>NS</td>
</tr>
</tbody>
</table>

For statistical analysis, the Student t test, the Mann-Whitney U test, and the Fisher exact test were used when appropriate (see Methods). Mean arterial pressure (MAP sleep) and blood pressure (BP sleep) during sleep or from 2 am until 6 am (MAP 2 to 6 AM, BP 2 to 6 AM) and minimal blood pressure during night (BP min) are given in mm Hg; plasma concentrations of sodium ([Na+]), potassium ([K+]), calcium ([Ca+]), and phosphate ([Pi]) are given in mmol/L; respective fractional excretions (FE) in % of filtered load; and urinary excretions (UV) in mmol/24 h or mg/24 h as indicated. P for differences between ClC-Kb/ClC-Kb and carriers of ClC-Kb T481S.
able hypertension.41,42 Again, those monogenic hypertensive disorders are rare. In contrast, the CIC-Kb
mutation is frequent and may well substantially contribute to the development of essential hypertension.

The impact of CIC-Kb
mutation is apparently modest and the mutation does not invariably lead to hypertension. Accordingly,
the development of hypertension in carriers depends on other genes and lifestyle.3,43 In this line, it is tempting to speculate that the enhanced prevalence of the gain of function mutation in an African population is the result of evolutionary pressure in a hot environment favoring enhanced loss of water and electrolytes through sweat. Thus, in a hot climate evolution selects individuals with enhanced ability to retain salt. In a cold environment with excessive salt supply those individuals are, however, at enhanced risk to renal salt retention, extracellular volume expansion, and volume hypertension, which indeed has been shown for Africans exposed to salt-rich Western diet.44

Subtle differences could be identified in renal function. The moderate but significant decrease of glomerular filtration rate may result from enhanced CIC-Kb channel activity, because CIC-Kb is expressed in the macula densa where Cl– reabsorption is a critical determinant of tubuloglomerular feedback.45 The increased Na+ plasma concentration may have resulted from an impaired ability of the kidney to eliminate Na+, which may be partially caused by decreased GFR. The hypernatremia was not likely caused by enhanced salt intake, because urinary Na+ excretion was rather decreased. The significantly enhanced fractional excretion of K+ may simply reflect the necessity to excrete a normal daily load of K+ at a decreased GFR.

Perspectives

Our data suggest that the enhanced activity of the CIC-Kb
channel indeed leads to renal salt retention and increase of blood pressure. Thus, we hypothesize that the CIC-Kb
mutation is a common genetic factor predisposing to the development of essential hypertension. The strength of the hypothesis is the doubtless profound functional significance of the mutation in vitro, suggesting a strong impact of the mutation on renal NaCl reabsorption in vivo. As a matter of fact, the mild phenotype of CIC-Kb
 carriers may be surprising in light of the profound impact of the mutation on channel function. The limitation of the hypothesis is the relatively small number of individuals studied. Thus, further studies in other populations are needed to confirm the association between the mutation and blood pressure, GFR, and renal handling of Na+. Specifically, it will be interesting to explore the prevalence of the mutation in hypertensive patients and in patients with endstage renal failure, the association of the mutation with blood pressure in Africans, and the influence of the mutation on sensitivity to salt intake and diuretic treatment. Moreover, additional studies may allow the identification of the molecular mechanism accounting for the enhanced activity of CIC-Kb
. Finally, the present observations raise the question why evolution did not lead to the preferential selection of the functionally more potent channel protein.

Acknowledgments

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References


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