ClC-K chloride channels: emerging pathophysiology of Bartter syndrome type 3

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Am J Physiol Renal Physiol 308: F1324–F1334, 2015. First published March 25, 2015; doi:10.1152/ajprenal.00004.2015.—The mutations in the CLCNKB gene encoding the ClC-Kb chloride channel are responsible for Bartter syndrome type 3, one of the four variants of Bartter syndrome in the genetically based nomenclature. All forms of Bartter syndrome are characterized by hypokalemia, metabolic alkalosis, and secondary hyperaldosteronism, but Bartter syndrome type 3 has the most heterogeneous presentation, extending from severe to very mild. A relatively large number of CLCNKB mutations have been reported, including gene deletions and nonsense or missense mutations. However, only 20 CLCNKB mutations have been functionally analyzed, due to technical difficulties regarding ClC-Kb functional expression in heterologous systems. This review provides an overview of recent progress in the functional consequences of CLCNKB mutations on ClC-Kb chloride channel activity. It has been observed that 1) all ClC-Kb mutants have an impaired expression at the membrane; and 2) a minority of the mutants combines reduced membrane expression with altered pH-dependent channel gating. Although further investigation is needed to fully characterize disease pathogenesis, Bartter syndrome type 3 probably belongs to the large family of conformational diseases, in which the mutations destabilize channel structure, inducing ClC-Kb retention in the endoplasmic reticulum and accelerated channel degradation.

Bartter syndrome; CLC family of chloride transporters and channels; chloride channel

Bartter Syndrome

BARTTER SYNDROME IS AN AUTOSOMAL recessive salt-wasting tubulopathy, characterized by hypokalemia, metabolic alkalosis and secondary hyperaldosteronism with normal-to-low blood pressure. Clinically, two variants are distinguished: antenatal Bartter syndrome (aBS) and classic Bartter syndrome (cBS) (8, 39, 48, 80, 81, 97). The aBS is a severe form leading to polyhydramnios due to polyuria in utero and premature birth. It is often complicated by dehydration episodes in the neonatal period and growth retardation. Nearly all patients present with hypercalciuria and medullary nephrocalcinosis. Elevated PGE2 production contributes to aggravate the course of the disease, a fact which prompted the alternate denomination of hyperprostaglandin E syndrome (81). aBS derives from mutations of the genes encoding the Na+-K+-2Cl- cotransporter and the Kir1.1 K+ channel (ROMK), SLC12A1, and KCNJ1, respectively (84–86). In the genetically based nomenclature, they correspond to Bartter syndrome type 1 (OMIM 601678) and 2 (OMIM 241200). cBS is usually diagnosed in early childhood. Failure to thrive is usually accompanied by low chloremia and severe hypokalemic alkalosis (8, 39, 81), polyuria and hypercalciuria being less frequent. cBS is due to mutations of the CLCNKB gene encoding the ClC-Kb Cl− channel (83). This corresponds to Bartter syndrome type 3 in the genetically based nomenclature (OMIM 607364). There is a large heterogeneity in Bartter syndrome type 3 presentation, extending from aBS to Gitelman syndrome (8, 38, 48). Gitelman syndrome is classically a defect in distal convoluted tubule (DCT) transport due to mutations in the SLC12A3 encoding the Na+-Cl− cotransporter (37, 97), but some patients carrying CLCNKB mutations have quite a similar phenotype, exhibiting hypomagnesemia, hypo- or normo-calciumia, insulin sensitivity to thiazide administration, and an absence of polyuria (8, 38).

The population of chloride channels in the renal tubule includes two additional players: ClC-Ka, a Cl− channel α-subunit of the same family as ClC-Kb, which is encoded by the CLCNKA gene, and the regulatory β-subunit barttin encoded by the BSND gene. A fourth variant of Bartter syndrome, aBS...
with sensorineural hearing loss (SNHL), is caused either by BSND mutations [OMIM 602522 (7) (see Ref. 21 for a review)], or by simultaneous mutations in the CLCNKB and CLCNKA genes (OMIM 613090) (69, 77).

**CIC-K Chloride Channels**

CIC-Ka and CIC-Kb (CIC-K1 and CIC-K2 in the rat and mouse) chloride channels were cloned in the 1990s by the groups of Uchida and Jentsch (44, 90, 95). They comprise 687 amino acids and are probably present as homodimers at the plasma membrane. Except for the rat CIC-K1 (95), functional expression of the CIC-Ks was unsuccessful until the cloning of barttin in 2001 (7), when functional studies established that barttin was a CIC-K regulatory subunit (19, 100). The electrophysiological properties of the CIC-Ks remain imperfectly known. CIC-Ka and rat and mouse CIC-K1 have been studied at the single-channel level and show conductances of 25–45 pS (3, 24, 43, 74) while it has not been possible until now to measure the unit conductance of CIC-Kb/CIC-K2. The only regulatory properties identified so far are the inhibition at acidic extracellular pH and the activation at high extracellular calcium (19, 30, 31, 100). In addition, a block at very basic 9–11 pH has also been reported (32). The physiological impact of these regulations remains uncertain. On the one hand, CIC-Ka and CIC-Kb are relatively insensitive to calcium between 0.5 and 2 mM (3, 33, 43), which makes it unlikely that calcium variations in the physiological range affect channel activity. On the other hand, pH changes such as those experienced during acidosis or alkalosis might affect CIC-Kb (pK\(_a\) = 7.9) (3) and CIC-Ka (pK\(_a\) = 7.3) (33).

At the protein level, the degree of identity between the two isoforms is slightly higher within (~80–90%) than across (human vs. rodent) species (44, 89, 94). This makes it impossible to determine homologs between species on the basis of sequence comparison. However, the diverse available data have led to the consensus that rodent CIC-K1 and human CIC-Ka, on the one hand, and rodent CIC-K2 and human CIC-Kb, on the other hand, are functional orthologs (89, 94).

Collectively, the two CIC-K proteins are present all along the distal nephron, from the thin ascending limb to the collecting duct (in the intercalated cells), but not in the proximal tubule (19). This is confirmed by barttin antibody staining the same segments of the renal tubule (19). The expression is restricted to the basolateral membrane (19, 89) except for the thin ascending limb (tAL) where CIC-K is present on both apical and basolateral membranes (96). The differential distribution of the two channels along the renal tubule has been difficult to ascertain in the absence of isoform-specific antibodies. However, immunocytochemistry performed on the kidneys of CIC-K1\(^{-/-}\) mice demonstrated that CIC-K1 was the only isoform present in the tAL (45) and that CIC-K2 was present in the thick ascending limb (TAL), DCT and the intercalated cells of the cortical collecting duct (CCD) (45). Patch-clamp experiments have also given some insight into the localization of the two channels: a recent study has demonstrated the correspondence between a 45-pS chloride channel in the mouse TAL (70) and recombinant mouse CIC-K1 (52). Thus a second 10-pS channel, activated by external calcium and at alkaline pH, which is expressed in the DCT and intercalated cells of the CNT and CCD, probably represents CIC-K2 (56, 66, 67, 70, 91). Together with results obtained with RT-PCR on rat renal segments (19, 44–46, 49, 96, 100), a consensus pattern emerges, which places CIC-K1 in the tAL and TAL, and CIC-K2 in the TAL, DCT, and the intercalated cells of the CNT and CD (Fig. 1A).

A major insight into CIC-Ka function was provided by Uchida and associates (1, 54, 61), who analyzed the consequences of CLCNK1 deletion in the mouse. Matsumura et al. (61) observed no hypokalemic alkalosis in Clcnk1\(^{-/-}\) mice, which would have been suggestive of Bartter syndrome, but polyuria (×5 compared with wild-type), associated with low urine osmolality (3-fold lower than WT), was dramatic. Furthermore, Clcnk1\(^{-/-}\) mice failed to concentrate urine after 24-h water deprivation or intraperitoneal injection of dDAVP (~2,500 mosmol/kgH\(_2\)O in WT mice vs. ~850 mosmol/kgH\(_2\)O in Clcnk1\(^{-/-}\) mice) (61). A follow-up study showed that the fractional excretion of sodium, chloride, and urea, as well as the total osmolar clearance, were not altered in Clcnk1\(^{-/-}\) mice (1). Thus polyuria is due to water diuresis, not osmotic diuresis. In addition, the urea and NaCl contents in the interstitium of the inner medulla were decreased by a factor of two in Clcnk1\(^{-/-}\) compared with Clcnk1\(^{+/+}\) mice (1). Furthermore, no Cl\(^{-}\) conductance was detected in microperfused tALs of CIC-K1\(^{-/-}\) mice (54, 61). Thus the loss-of-function of CIC-K1 causes nephrogenic diabetes insipidus by impairing the countercurrent system in the inner medulla (1). As pointed out by Matsumura et al. (61), it is doubtful that CLCNKA deletion would cause a similar pathology in humans, who have a less developed inner medulla.

The results of Uchida’s group (1, 54, 61) confirm that CIC-K1 is mostly present in the TAL. Thus, although no Clcnk2\(^{-/-}\) mouse line is yet available, we may speculate that CIC-K2 has a predominant role in the other parts of the mouse renal tubule. Ion transport models for the TAL and DCT (Fig. 1, B and C) involve two distinct Na\(^{+}\)/Cl\(^{-}\) cotransporters, NKCC2 and NCC, respectively, at the apical membrane. The exit of chloride on the basolateral side is dependent on Cl\(^{-}\) channels (18, 39, 79). Basolateral Cl\(^{-}\) channels are also important for ion transport in the intercalated cells (18, 79, 88) (Fig. 1D).

Classically, the β-intercalated cells participate in bicarbonate secretion but Eladari and Chambrey (11, 18, 51) recently discovered a novel, neutral pathway for NaCl absorption in these cells (Fig. 1D), which includes Cl\(^{-}\) conductance at the basolateral side (18, 79, 88). The α-intercalated cells (Fig. 1D) participate in acid-base balance by secreting protons via the V-type H\(^{+}\) ATPase at the apical membrane and the AE1 HCO\(_3\)/Cl\(^{-}\) exchanger at the basolateral membrane. Basolateral Cl\(^{-}\) channels optimize H\(^{+}\) secretion by recycling Cl\(^{-}\), thus facilitating the functioning of the AE1 exchanger (88). In summary, CIC-K2 is expected to play a key role in Cl\(^{-}\) absorption in the DCT, CNT/CCD, and to a lower extent in the TAL, where CIC-K1 is present (52, 70). There has been yet no patch-clamp study of CIC-K chloride channels in the α-intercalated cells, keeping open the possibility that the two channels cooperate at this site.

**Structure of CIC-K Chloride Channels**

Renal chloride channels belong to the channel subgroup of the CIC family of chloride channels and transporters, which also includes CIC-1 and CIC-2 (21, 42, 93). The structure of this protein family was revealed in 2002 with the crystalliza-
Fig. 1. Localization of the 2 ClC-K channels along the rodent renal tubule and ion transport systems in various parts of the distal nephron. A: localization of ClC-K1 (blue) and ClC-K2 (orange) along the nephron. The localization is based on RT-PCR of rat isolated renal segments (49, 66, 100), immunochemistry (19, 45), and patch-clamp studies of the mouse renal tubule (50, 56, 66, 67, 70). The distribution pattern remains incomplete: 1) in the absence of a dedicated patch-clamp study, it is not known whether ClC-K1 is present in the α-intercalated cells together with ClC-K2; 2) although, ClC-K1 messenger RNA is present in the late distal tubule and the collecting duct, ClC-K1 has not been detected in these two renal segments using the patch-clamp technique (50, 56, 66, 67, 70).

CIC-K1 might be active only during specific physiological conditions. B: NaCl absorption in the thick ascending limb involves Na+/K+/2Cl⁻ cotransport (NKCC2) in the apical membrane and ClC-K2 and ClC-K1 in the basolateral membrane. C: NaCl absorption in the distal convoluted tubule involves Na+/Cl⁻ cotransport (NCC) in the basolateral membrane and ClC-K2 in the basolateral membrane. D: β-intercalated cells (β ic) absorb NaCl (18), and the α-intercalated cells (α ic) secrete protons. NaCl absorption involves the Pendrin Cl⁻/HCO₃⁻ exchanger and NDCBE Na⁺-driven Cl⁻/HCO₃⁻ exchanger in the apical membrane, and AE4 Cl⁻/HCO₃⁻ exchanger and ClC-K2 in the basolateral membrane. The KCC4 K⁺-Cl⁻ cotransporter might also allow Cl⁻ exit. The α-intercalated cells secrete H⁺ by the operation of the V-type H⁺-ATPase in the apical membrane and AE1 Cl⁻/HCO₃⁻ exchanger in the basolateral membrane. ClC-K2 with or without ClC-K1 is present in the basolateral membrane and might optimize the turnover rate of the exchanger. See the text for additional definitions.

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likely to be conserved among the eukaryotic CIC (62). Several studies reported a regulatory role of binding of adenine nucleotides to CBS domains in ClC-1, ClC-2, and ClC-5 (65, 92, 105). This part of the protein is known to be involved in the regulation of common gating (9, 20). A similar role has yet to be demonstrated for the ClC-Ks.

As noted above, the ClC-K currents recorded in overexpression systems are inhibited at acid pH and activated by an increase in extracellular Ca\(^{2+}\) concentration (19, 30, 31, 100). A detailed screening of acidic amino acid residues led to the identification of two critical amino acids (E261 and D278, localized in the extracellular loop between \(\alpha\)-helices I and J), which form the putative extracellular Ca\(^{2+}\)-binding site (31). A histidine residue on position 497 at the beginning of \(\alpha\)-helix Q is responsible for the H\(^+\)-induced block (30) (Fig. 2).

**CLCNKB Mutations in Bartter Syndrome**

Since the pioneering work of Simon et al. (83) identifying mutations in the **CLCNKB** gene as a cause of Bartter syndrome type 3, a number of publications have reported pathogenic mutations (3, 8, 26, 43, 48, 50, 76, 83, 102, 103). As a whole, according to the Human Gene Mutation Database (free access HGMD database, Biobase International, www.hgmd.cf.ac.uk), >54 mutations have been described, including complex rearrangements, large and small deletions, small insertions, nonsense or missense mutations, and splice site mutations. One characteristic feature of the disease is the frequent occurrence of total **CLCNKB** gene deletion (8, 29, 38, 48, 83).

The 50 point mutations (missense and nonsense) illustrated in Fig. 3 and summarized in Table 1 are distributed all along the protein with no clear hotspots. Several mutations are located close to several parts of the selectivity filter, in the C-D linker and \(\alpha\)-helix D (3 mutations), E-F linker and \(\alpha\)-helix F (2 mutations), and \(\alpha\)-helix N (8 mutations), respectively. By contrast, no mutations were reported in the vicinity of H\(^+\) and Ca\(^{2+}\)-binding sites. Altogether, 60% of the mutations are located on \(\alpha\)-helices, 20% in helix linkers, and 20% in the cytoplasmic part of the protein. About 16 homozygous muta-
tions have been found in patients (A77P, L81P, T115P, P124L, G164C, V170M, A204T, G246R, S297R, R351W, H357Q, G424E, R438H, K560M, R595X, W610X). A few mutations such as A204T (29, 76, 83), P124L (3, 5, 43, 48, 83), R438H (3, 5, 43, 48, 83), and W610X (14, 50, 68) have been reported independently by several authors. In particular, A204T, a founder mutation in Spain, is the prevalent mutation in this country (28).

Functional Consequences of CLCNKB Mutations

Only 20 CLCNKB mutations have been functionally analyzed. This low figure is mainly due to technical problems regarding ClC-Kb functional expression. Current recording of ClC-Kb in *Xenopus laevis* oocytes or cultured cells has remained difficult even after the discovery of the barttin regulatory subunit: ClC-Kb levels of current are fivefold lower than those elicited by ClC-Ka (36, 43). An additional, specific problem concerns Western blotting of the ClC-K protein: overexpressed ClC-K proteins tend to form stable aggregates so that the band corresponding to the monomeric protein is often hardly visible (10, 43, 100, 101; also see Ref. 36).

Before the discovery of barttin, Waldegger et al. (101) succeeded in recording chloride currents from a chimeric human ClC-Kb/rat ClC-K1 construct [ClC-Kb (c)] and analyzed a selection of mutations reported by Simon et al. (83) in *X. laevis* oocytes using two-electrode voltage-clamp: P124L, A204T, A349D, and R438C abolished ClC-Kb (c) current while Y432H dramatically reduced it, by at least fourfold. Obviously, a limit of this interesting study lies in the use of chimeric channels, as it is difficult to anticipate how the presence of rat ClC-K1 stretches in the ClC-Kb protein might influence the observed effects. Soon afterward, Estevez et al. (19) recorded for the first time ClC-K/barttin complexes in *X. laevis* oocytes. They tested several of the mutations reported by Simon et al. (83) and Konrad et al. (48) by coinjecting mRNA for several ClC-Kb mutants with Y98A-mutant barttin to increase surface expression of the channel complex. Overall, A349D and R438C mutations totally abolished currents, as in the above-mentioned study, while P124L, A204T, R538P, and K560M mutations decreased the currents by 60–80%. More recently, Yu et al. (102) analyzed R30X, A210V, and R351W mutations by the same methods. As expected, the R30X mutant generated no current; A210V and R351W currents were reduced by 53 and 39%, respectively. Overall, all these mutations (except A210V and R351W) reduced channel currents by >60% (19, 102).

Our group has recently investigated eight novel CLCNKB mutations (L81P, R92W, G120R, V170M, G246R, R351P, G424E, and L439P) (3, 43). We also selected some published
defect behind the development of Bartter syndrome type 3 mutations to gain a deeper understanding of the molecular

<table>
<thead>
<tr>
<th>Mutation Number</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Protein Domain</th>
<th>Remaining Current</th>
<th>Sensitivity To H+ and Ca2+</th>
<th>Surface Expression</th>
<th>Total CIC-Kb Protein</th>
</tr>
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<tbody>
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<td>1</td>
<td>c.88C&gt;T (102)</td>
<td>R30X</td>
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<td>2</td>
<td>c.216C&gt;A (6)</td>
<td>A61D</td>
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<td>R76X</td>
<td>α-Helix B</td>
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<td>4</td>
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<tr>
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<td>A77T</td>
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<tr>
<td>6</td>
<td>c.242T&gt;C (36)</td>
<td>L81P</td>
<td>α-Helix B</td>
<td>35% (43)</td>
<td>30% (43)</td>
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<tr>
<td>7</td>
<td>c.274C&gt;T (43)</td>
<td>R92W</td>
<td>α-Helix C</td>
<td>67% (43)</td>
<td>85% (43)</td>
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<td>8</td>
<td>c.343A&gt;C (3)</td>
<td>T115R</td>
<td>α-Helix C</td>
<td>18% (3)</td>
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<td>c.358G&gt;C (3)</td>
<td>G120R</td>
<td>C-D linker</td>
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<td>G120V</td>
<td>C-D linker</td>
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<td>11</td>
<td>c.371C&gt;T (48, 83)</td>
<td>P124L</td>
<td>α-Helix D</td>
<td>0% (101); 45% (19); 73% (3)</td>
<td>Altered (3)</td>
<td>= (101)</td>
<td>= (101)</td>
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<td>c.451T&gt;C (83)</td>
<td>L139P</td>
<td>D-E linker</td>
<td>27% (3)</td>
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<td>V149E</td>
<td>α-Helix E</td>
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<td>G164C</td>
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<tr>
<td>15</td>
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<td>V170M</td>
<td>α-Helix F</td>
<td>60% (3)</td>
<td>Altered (3)</td>
<td>60% (3)</td>
<td>= (77)</td>
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<tr>
<td>16</td>
<td>c.610G&gt;A (76, 83)</td>
<td>A204T</td>
<td>α-Helix G</td>
<td>25% (19); 0% (101)</td>
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<tr>
<td>17</td>
<td>c.629C&gt;T (102)</td>
<td>A210V</td>
<td>α-Helix G</td>
<td>approximately 50% (102)</td>
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<td>G246R</td>
<td>α-Helix J</td>
<td>10%* (43)</td>
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<td>K-L linker</td>
<td>63% (43)</td>
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<td>65% (43)</td>
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<td>R351W</td>
<td>K-L linker</td>
<td>approximately 60% (102)</td>
<td>Ca2+ altered (102)</td>
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<td>34</td>
<td>c.1294T&gt;C (83)</td>
<td>Y432H</td>
<td>α-Helix N</td>
<td>−20% (101)</td>
<td>None (43)</td>
<td>101)</td>
<td>(101)</td>
</tr>
<tr>
<td>35</td>
<td>c.1309G&gt;A (51)</td>
<td>G437C</td>
<td>α-Helix N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>c.1312C&gt;T (83)</td>
<td>R438C</td>
<td>α-Helix N</td>
<td>0% (101); 10%* (43)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>c.1313G&gt;A (48, 103)</td>
<td>R438H</td>
<td>α-Helix N</td>
<td>18%* (43)</td>
<td>None (43)</td>
<td>101)</td>
<td>(101)</td>
</tr>
<tr>
<td>38</td>
<td>c.1316T&gt;C (43)</td>
<td>L439P</td>
<td>α-Helix N</td>
<td>15%* (43)</td>
<td>None (43)</td>
<td>101)</td>
<td>(101)</td>
</tr>
<tr>
<td>39</td>
<td>c.1325A&gt;G (29)</td>
<td>E442G</td>
<td>α-Helix N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>c.1340T&gt;C (99)</td>
<td>I447T</td>
<td>N-O linker</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>41</td>
<td>c.1409G&gt;A (53)</td>
<td>G470E</td>
<td>α-Helix O</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>c.1537C&gt;T (83)</td>
<td>Q513X</td>
<td>α-Helix Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>c.1648G&gt;C (31)</td>
<td>R538P</td>
<td>Liner R-CBS1</td>
<td>25% (19)</td>
<td></td>
<td></td>
<td>Ca2+ altered (59)</td>
</tr>
<tr>
<td>44</td>
<td>c.1714A&gt;T (31)</td>
<td>K560M</td>
<td>CBS1</td>
<td>50% (19)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>c.1685C&gt;T (27)</td>
<td>M562T</td>
<td>CBS1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>c.1733T&gt;A (48)</td>
<td>S573Y</td>
<td>CBS1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>c.1732G&gt;A (27)</td>
<td>K578E</td>
<td>CBS1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>c.1783C&gt;T (6)</td>
<td>R595X</td>
<td>CBS1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>49</td>
<td>c.1830G&gt;A (26, 51)</td>
<td>W610X</td>
<td>CBS1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>c.1877G&gt;A (99)</td>
<td>C626Y</td>
<td>CBS2</td>
<td></td>
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*Residual current not statistically different from measured current in noninjected oocytes (36). Numbers in parentheses indicate references.

mutations to gain a deeper understanding of the molecular defect behind the development of Bartter syndrome type 3 (T115P, P124L, L139P, and R438H) (8, 48, 83, 103). In these studies, we coexpressed mutant CIC-Kb proteins with wild-type barttin in X. laevis oocytes or HEK293 cells. The currents carried by the CIC-Kb mutants were reduced to different extents. For nearly half of the mutants (3, 43), no current was detected (T115P, G246R, G424E, R438H, and L439P). Four mutants showed 50–70% residual activity (R92W, P124L, V170M, R351P), and two had a low activity of ~20–40% (L81P, L139P, G120R) (3, 43). Note that in our hands, P124L generated a noticeable current (40%) while lower currents (0–20%) were detected in previous studies (48, 85).

Surface expression varied from 0 to 85% compared with WT CIC-Kb and was proportional to current level, indicating that the primary defect shown by mutants concerns the number of channel units in the membrane (3, 43). For mutants generating sufficient current, we evaluated unit conductance (R92W, R351P), anion selectivity (L81P, R92W, V170M, and R351P), and pH and Ca2+ sensitivity (L81P, R92W, R351P, P124L, and V170M) (3, 43). There was no change in conductance, selectivity, or regulation except for two mutants (P124L, V170M) that displayed a dramatic alteration in pH sensitivity (see below). These results go along with the hypothesis that the primary defect induced by mutations is generally an alteration in surface expression of the channel.
Altogether, among the 20 mutations analyzed for the total current, 40% do not show any detectable activity (T115P, G246R, A349D, G424E, G424R, R438C, R438H, and L439P), 40% maintain >50% activity (R92W, G120R, P124L, V170M, A210V, R351P, R351W, and K560M), and 20% have a smaller activity of ~20–30% (L81P, L139P, A204T, and R538P). Total protein abundance (considering bands corresponding to monomeric and dimeric proteins) was assayed in HEK293 cells for a minority of mutants (43). Protein abundance was clearly reduced for those mutants that showed no current and no surface expression (G246R, G424E, R438H, and L439P). This strongly suggests that the produced transcript is not stable in the expression system. Protein abundance was not reduced for mutants showing >50% activity (R92W and R351P) nor for one mutant with 20–30% activity (L81P). This suggests that the stability of the protein (for instance, accelerated retrieval from the membrane) in the membrane could be decreased.

Mutations that alter regulation/conduction appear to be predominant in myotonia caused by mutations in the CLCN1 gene: ClC-1 is a voltage-dependent channel, which is activated by depolarization; many mutations profoundly shift voltage dependence toward more positive voltages (71, 73) or even convert ClC-1 to an inwardly rectifying channel (22, 104). By contrast, mutations altering channel gating are not frequent in Bartter disease type 3. We have evaluated pH and Ca$^{2+}$ sensitivity for six ClC-Kb mutants and found alterations in only two (3, 43), while studies by others detected two additional mutants showing some kind of altered regulation. Maduke et al. (59) reported that the R538P mutation totally abolished sensitivity to Ca$^{2+}$. This is associated with a clear reduction in current (19). According to the authors, arginine 538 being located in the cytoplasm after helix R, the lack of sensitivity to Ca$^{2+}$ implies a long-distance effect from the cytoplasmic side of the protein to its extracellular side (59). Similar Ca$^{2+}$ independence was reported by Yu et al. (102) for the R351W mutation (at the outer aspect of the protein in K-L linker), which was also associated with a 60% decrease in current.

In our hands, two mutations (V170M and P124L) dramatically altered pH and Ca$^{2+}$ sensitivity (3). The curve of H$^{+}$-dependent inhibition shifted by ~1.5 pH units toward more acid values for V170M and P124L mutants compared with WT ClC-Kb (pK_H: 6.0 for V170M and 6.1 for P124L vs. 7.6 for WT). Our results suggest that these mutations do not alter directly the binding site of H$^{+}$ but disrupt the gating function of the channel, thus indirectly affecting the sensitivity to pH_{ext} (3). As a direct consequence, the mutant ClC-Kb channels are maximally active at pH 7.4 while the activity of WT ClC-Kb at this pH is only 20% of its maximal activity. In addition, we observed that the mutants were completely independent of Ca$^{2+}$ at pH 7.4, perhaps because they are maximally active at this pH (3). In parallel, there was a clear reduction in current and surface expression in the two mutants (3). Therefore, these mutations result in hyperactive channels, which tend to offset the functional impact of reduced expression at the membrane. The opposite mechanism might operate in the case of T481S ClC-Kb, a CLCNKB single-nucleotide polymorphism that has been linked to essential hypertension (41, 82). Jeck et al. (40) showed that the 5- to 20-fold increase in ClC-Kb current induced by the T481S mutation was associated with higher sensitivity to pH (lower activity at pH 7.4) and an increase in membrane channel expression, a mirror image of what is observed in Bartter syndrome for ClC-Kb mutants.

**Alteration of Functional Motifs as a Cause of Bartter Syndrome**

Several of the mutations that have been functionally analyzed are located around the selectivity filter. Three mutations in α-helix N (R438C, R438H, L439P) and one close to it (G424E) are associated with a reduction in total protein abundance, an absence of expression at the membrane, and an absence of current. Two mutations (P124L and V170M) alter channel regulation. These results strongly suggest that the selectivity filter is of paramount importance for structure stability and gating. The hyperactivity of P124L and V170M is puzzling when considered in the context of disease pathophysiology. As already mentioned, mutations of the CLCN1 gene causing myotonia often result in gating alterations. However, these alterations always render the channel less active (71). Thus it is currently difficult to appreciate whether the uncoupling of channel activity from pH- and Ca$^{2+}$-dependent gating is a coincidental side effect of the mutations or whether these mutations alter yet unidentified regulators of channel activity.

By contrast, the available data provide little information about the functional effects of CLCNKB mutations in two other regions: α-helices B and J (8 mutations), which interact with barttin, and CBS domains (7 mutations). The CBS domains might be particularly critical for channel function. They participate in ClC common gating (20, 25), likely by sensing intracellular metabolites (35). They also participate in channel trafficking and targeting to the plasma membrane. The partial deletion of the cytoplasmic domain leads to a retention of ClC-0 and ClC-1 proteins into the endoplasmic reticulum (ER) (20, 34, 57, 78). The only CBS1-located mutation that has been functionally characterized in overexpression systems (K560M) reduces ClC-Kb current to 25% of control (19).

The interface between the two monomers, mainly composed of α-helices H, I, P, and Q, is potentially another critical region. In the case of Dent’s disease, an X-linked hereditary disease due to mutations in the CLCN5 gene encoding the Cl$^{-}$/H$^{+}$ exchanger CIC-5, ~60% of the mutations are located at the dimer interface (55). These mutations either result in ER retention or channel dysfunction (55). Mutations at the same location are also frequent in the CLCN7 gene causing osteopetrosis, or in the CLCN1 gene, where they modify channel gating (71). Unexpectedly, only three mutations have been found at the dimer interface in Bartter disease: P216L (50) and A242E (5), which have not been functionally investigated, and G246R, which shows no current, no surface expression, and reduced protein abundance (43). The latter result suggests a destabilization of the protein.

**Phenotype-Genotype Relationship**

The first papers reporting mutations in the CLCNKB gene causing Bartter syndrome type 3 already mentioned that the
severity of the disease was highly variable (38, 48, 83). In particular, whole gene deletion, which is frequent in this disease, may cause aBS or cBS (38, 48, 83). This is also the case for the A204T and R438H mutations (29, 76, 103). The R438H mutation results in a nonfunctioning channel (43), and A204T reduces current to 0–25% of control (48, 83). In particular, Zelikovic et al. (103) showed that patients carrying the mutation R438H within one same large family could present with mild or severe symptoms. Especially in the case of CLCNKB gene deletion, the variable severity of the disease suggests that CIc-Ka Cl\(^-\) channel, K\(^+\)-Cl\(^-\) cotransporter (8) or additional Cl\(^-\) channels (60) might be expressed in the TAL in a variable fashion and compensate for the loss of function of CIc-Kb in some patients. In the case of point mutations, one alternative possibility would be that the defect being due to conformational destabilization has a variable impact depending on the individual.

The eight patients homozygous for V170M, or compound heterozygous with another mutation (whole gene deletion or mutations resulting in the production of unstable mRNAs or truncated proteins) have a mild phenotype (3). It is likely that these patients mainly express V170M homodimers of this hyperactive channel with reduced expression at the membrane. This is also the case for one patient homozygous for P124L, but not for two other patients compound heterozygous for P124L and G465R (3). It will be interesting to observe whether more mutations of this type are detected in the future and whether they are associated with a mild presentation.

**Conclusion**

Overall, currently available data suggest that CLCNKB mutations might be arranged in two main classes: (1) mutations causing fully impaired (~40% of the mutations) or reduced (~60% of the mutations) channel expression at the membrane; and (2) mutations combining reduced channel surface expression with altered channel properties (~20% of the total number of mutations analyzed).

It is clear that further investigation is needed to fully characterize the pathogenesis of Bartter disease type 3, which has been hampered by technical difficulties in the expression of this particular chloride channel. However, Bartter disease type 3 probably belongs to the large family of conformational diseases, in which the mutations destabilize channel structure, inducing retention in the ER and accelerated channel degradation. Accordingly, a classic approach to treatment would be to test the efficacy of adapted chaperons for enhancing CIc-Kb surface expression. An approach of this type has been recently performed with some success on W610X CIc-Kb mutants in MDCK cells using an aminoglycoside derivative (14). This long-term strategy might be helpful for those patients who present with a severe form of Bartter syndrome type 3.

At the end of this review dedicated to the pathophysiology of Bartter syndrome, it is worth noting that one CLCNKB single-nucleotide polymorphism (T481S) has been linked to essential hypertension (41, 82). Although the association with hypertension was not replicated by some studies (23, 47, 87), the idea that CIc-Kb might be functionally important for long-term control of blood pressure remains attractive, and is in line with recent findings pointing out the importance of chloride in blood pressure regulation (18). In addition, several CLCNKA single-nucleotide polymorphisms have been associated with salt-sensitive hypertension (4) or heart failure (10). Altogether, these various reports suggest that the pathophysiological impact of CIc-Kb and CIc-Ka on NaCl balance might be not restricted to Bartter syndrome.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: O.A. and J.T. contributed conception and design of work; O.A., M.K., R.B., S.L., R.V.-P., and J.T. provided the data; O.A. and J.T. analyzed the data; O.A. and J.T. interpreted the results of the study; O.A. and J.T. drafted the manuscript; O.A., M.K., R.B., S.L., R.V.-P., and J.T. edited and revised the manuscript; O.A., M.K., R.B., S.L., R.V.-P., and J.T. approved final version of manuscript.

**REFERENCES**


AJP-Renal Physiol • doi:10.1152/ajprenal.00004.2015 • www.ajprenal.org


Urbanova M, Reiterova J, Stekrova J, Lnenicka P, Rysava R. DNA analysis of renal electrolyte transporter genes among patients suffering

Downloaded from http://ajprenal.physiology.org/ by 10.220.32.246 on June 17, 2017

AJP-Renal Physiol • doi:10.1152/ajprenal.00004.2015 • www.ajprenal.org
from Bartter and Gitelman syndromes: summary of mutation screening. 

Debaix H, Grisart B, Bridoux F, Unwin R, Moulin B, Haymann JP, 
Vantyghem MC, Rigothier C, Dussol B, Godin M, Nivet H, Dubourg 
L, Tack I, Gimenez-Roqueplo AP, Houllier P, Blanchard A, Devuyst 
O, Jeunemaitre X. Spectrum of mutations in Gitelman syndrome. J Am 

Konrad M, Seyberth HW. Barttin increases surface expression and changes 

101. Waldegger S, Jentsch TJ. Functional and structural analysis of ClC-K chloride 

Identification and functional analysis of novel mutations of the CLCNKB 
gene in Chinese patients with classic Bartter syndrome. Clin Genet 77: 

103. Zelikovic I, Szargel R, Hawash A, Labay V, Hatib I, Cohen N, 
Nakhoul F. A novel mutation in the chloride channel gene, CLCNKB, 
as a cause of Gitelman and Bartter syndromes. Kidney Int 63: 

104. Zhang J, Sanguinetti MC, Kwiecinski H, Ptacek LJ. Mechanism of 
inverted activation of ClC-1 channels caused by a novel myotonia 

105. Zifarelli G, Pusch M. Intracellular regulation of human ClC-5 by 