Hypokalaemic salt-losing tubulopathies: an evolving story

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Introduction

Bartter syndrome, first described in 1962 [1], is a group of closely related hereditary tubulopathies. All variants of the syndrome share several clinical characteristics including renal salt wasting, hypokalaemic metabolic alkalosis, hyperreninaemic hyperaldosteronism with normal blood pressure, and hyperplasia of the juxtaglomerular apparatus [2–4]. All forms of the syndrome are transmitted as autosomal recessive traits.

Three distinct clinical phenotypes have been distinguished, including antenatal Bartter syndrome, classic Bartter syndrome and Gitelman syndrome (Table 1). Recently, however, phenotypic overlap has been noted, and additional variants of Bartter syndrome have been described (Table 1, Figure 1), thereby expanding the clinical spectrum of the syndrome and providing further insight into the pathophysiological mechanisms underlying this complex disorder. Over the past decade, the breakthrough in molecular biology and molecular genetics has produced the tools to investigate various forms of Bartter syndrome at the molecular level. As a result, exciting discoveries have been made and the underlying molecular defects in these variants have been defined. The molecular studies of Bartter syndrome have been important not only in clarifying the genetic basis of this tubulopathy, but also in providing new and important insight into the function of specific transport proteins and into the physiology of renal tubular reclamation of solutes.

Phenotypes

Traditionally, the disease has been classified into three distinct phenotypes (Table 1). First, antenatal Bartter syndrome, also known as hyperprostaglandin E syndrome, is the most severe form of the disease. It is characterized by polyhydramnios, premature birth, life-threatening episodes of salt and water loss in the neonatal period, hypokalaemic alkalosis and failure to thrive, as well as hypercalciuria and early-onset nephrocalcinosis [4,5]. Secondly, classic Bartter syndrome occurs in infancy or early childhood. It is characterized by marked salt wasting and hypokalaemia leading to polyuria, polydipsia, volume contraction, muscle weakness and growth retardation. Hypercalciuria and nephrocalcinosis may occur [2,3]. Thirdly, Gitelman syndrome is characterized by a mild clinical presentation in older children or adults [6]. Patients may be asymptomatic and present with transient muscle weakness, abdominal pain, symptoms of neuromuscular irritability or unexplained hypokalaemia. Hypocalciuria and hypomagnesaemia are typical [4,6].

Recently, an additional variant of antenatal Bartter syndrome associated with sensorineural deafness, renal failure and typical appearance has been described (Table 1) [7]. Finally, in 2002, several patients with severe autosomal dominant hypocalcaemia associated with Bartter syndrome were reported (Table 1) [8,9]. Aside from their deficient parathyroid hormone secretion and renal Ca²⁺ and Mg²⁺ wasting, these patients also have impaired tubular Cl⁻ reabsorption, negative NaCl balance, hyperaldosteronism and marked hypokalaemia.

Pathogenesis

The pathogenesis of these pathophysiologically complex and heterogeneous disorders has been the subject of intensive research for the past four decades. Nevertheless, as late as 1995, the molecular basis of
**Table 1. Variants of Bartter syndrome**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Defective gene (genotype)</th>
<th>Locus</th>
<th>Defective transporter/protein</th>
<th>Location</th>
<th>OMIM no. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antenatal</td>
<td>SLC12A1 (type I) or KCNJ1 (type II)</td>
<td>15q15-21 or 11q24-25</td>
<td>Na(^+)-K(^+)-2Cl(^-) co-transporter, NKCC2 or K(^+) channel, ROMK</td>
<td>TAL</td>
<td>241200 or 601678</td>
</tr>
<tr>
<td>Classic</td>
<td>CLCNKB (type III)</td>
<td>1p36</td>
<td>Cl(^-) channel, ClC-Kb</td>
<td>TAL</td>
<td>602023</td>
</tr>
<tr>
<td>Gitelman</td>
<td>SLC12A3 or CLCNKB</td>
<td>1q13 or 1p36</td>
<td>Na(^+)–Cl(^-) co-transporter, TSC (NNCT) or Cl(^-) channel, ClC-Kb</td>
<td>DCT</td>
<td>263800</td>
</tr>
<tr>
<td>BSND</td>
<td>BSND (type IV)</td>
<td>1p31</td>
<td>Barttin (β subunit of ClC-Ka/ClC-Kb)</td>
<td>TAL/inner ear</td>
<td>602522</td>
</tr>
<tr>
<td>ADH</td>
<td>CASR</td>
<td>3q13.3-q21</td>
<td>Calcium-sensing receptor (CaSR)</td>
<td>TAL</td>
<td>601198</td>
</tr>
</tbody>
</table>

TAL, thick ascending limb of the loop of Henle; DCT, distal convoluted tubule; BSND, Bartter syndrome with deafness; ADH, autosomal dominant hypocalcaemia.


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**Fig. 1.** Transport pathways in the thick ascending limb of the loop of Henle (A) and the distal convoluted tubule (B). (A) Cl\(^-\) reabsorption across the luminal membrane occurs via the Na\(^+\)-K\(^+\)-2Cl\(^-\) co-transporter (NKCC2). This co-transporter is driven by the low intracellular Na\(^+\) and Cl\(^-\) concentrations generated by the basolateral Na\(^+\)-K\(^+\)-ATPase and ClC-Kb, respectively. In addition, ROMK enables functioning of NKCC2 by recycling K\(^+\) back to the lumen. The lumen-positive electrical potential, which is generated by Cl\(^-\) entry into the cell and K\(^+\) exit from the cell, drives paracellular Ca\(^{2+}\) and Mg\(^{2+}\) transport from lumen to blood. Activation of the basolateral calcium-sensing receptor (CaSR) inhibits the luminal ROMK channel which, in turn, results in decreased NaCl reabsorption and (secondary to the reduction in the intraluminal positive potential) increased urinary Ca\(^{2+}\) and Mg\(^{2+}\) excretion. (B) Cl\(^-\) transport occurs via the luminal, thiazide-sensitive NaCl co-transporter (TSC). Cl\(^-\) exit to blood is mediated by basolateral Cl\(^-\) channels. Ca\(^{2+}\) and Mg\(^{2+}\) enter the cell via luminal voltage-activated Ca\(^{2+}\) and Mg\(^{2+}\) channels and exit the cell via basolateral Na\(^+\)/Ca\(^{2+}\) and Na\(^+\)/Mg\(^{2+}\) exchangers. The depicted apical Mg\(^{2+}\) channel and basolateral Na\(^+\)/Mg\(^{2+}\) exchanger are putative. Variants of Bartter syndrome caused by defects in these transport mechanisms are depicted. BSND, Bartter syndrome with deafness; ADH, autosomal dominant hypocalcaemia.
Barter syndrome was still considered an ‘unsolved puzzle’ [10]. It has been suggested that Barter syndrome results from defective transepithelial transport of Cl\textsuperscript{–} in the thick ascending limb of the loop of Henle (TAL) and the distal convoluted tubule (DCT).

Transepithelial Cl\textsuperscript{–} transport in the TAL is a complex process that involves coordinated interplay between the luminal, bumetanide-sensitive Na\textsuperscript{+}–K\textsuperscript{+}–2Cl\textsuperscript{–} co-transporter (NKCC2), the luminal, ATP-regulated, inwardly rectifying K\textsuperscript{+} channel (ROMK), the basolateral Cl\textsuperscript{–} channel (CLC-Kb), as well as other co-transporters and channels (Figure 1A) [4,11,12]. Chloride is reabsorbed across the luminal membrane of the TAL cell by the activity of NKCC2. This co-transporter is driven by the low intracellular Na\textsuperscript{+} and Cl\textsuperscript{–} concentrations generated by the Na\textsuperscript{+}–K\textsuperscript{+}–ATPase and CLC-Kb, respectively. In addition, ROMK enables functioning of NKCC2 by recycling K\textsuperscript{+} back to the renal tubular lumen. Chloride transport in the distal convoluted tubule occurs primarily via the luminal, thiazide-sensitive NaCl co-transporter (TSC) (Figure 1B) [11,12]. Cl\textsuperscript{–} exit to blood is mediated via basolateral Cl\textsuperscript{–} channels.

**Genetic variants**

A series of genetic linkage and mutation detection studies by Lifton and co-workers [13–16] over the past several years have shown that defects in the genes encoding several transporters in the TAL and the DCT are responsible for various forms of Barter syndrome. The genetic variants that have been identified include the following (Table 1, Figure 1) [4,11–16].

(i) Barter syndrome type I is caused by mutations in the NKCC2 gene, *SLC12A1*. This gene belongs to the family of electroneutral chloride-coupled co-transporter genes [17] and resides on chromosome 15q15–21. This genetic variant leads to antenatal Barter syndrome [13,18,19]. Not surprisingly, this clinical phenotype is similar to the biochemical abnormalities induced by chronic furosemide therapy. The stimulation of prostaglandin production in the antenatal variant appears to be secondary to the defect in NaCl transport in the TAL.

(ii) Barter syndrome type II is caused by mutations in the ROMK gene (*KCNJ1*), that is located on chromosome 11q24–25. ROMK mutations lead to the clinical phenotype of antenatal Barter syndrome [14,20,21]. Interestingly, this genetic defect, which leads to dysfunction of ROMK and impaired K\textsuperscript{+} secretion not only in the TAL but also in the collecting duct, occasionally may result in transient neonatal hyperkalaemia and acidosis before the typical hypokalaemic alkalosis develops [22].

(iii) Barter syndrome type III is caused by mutations in the ClC-Kb gene (*CLCNKB*) [15]. This gene, which is located on chromosome 1p36, belongs to the family of genes encoding voltage-gated Cl\textsuperscript{–} channels, to which *CLCN5* (Dent’s disease gene) also belongs. Patients with *CLCNKB* mutations usually have classic Barter syndrome.

(iv) Gitelman syndrome is caused by mutations in the TSC (or NCCT) gene (*SLC12A3*) [16,23]. The TSC gene belongs to the family of genes encoding electroneutral chloride-coupled co-transporters [17] and resides on chromosome 16q13. Many of the symptoms of Gitelman syndrome are reminiscent of the effects of chronic thiazide administration.

Recent data, however, have suggested that the genotype–phenotype correlation is not so clear cut and that phenotypic overlap may occur. Konrad *et al.* [24] and Peters *et al.* [25] reported that mutations in *CLCNKB* can also cause phenotypes that overlap with either antenatal Barter syndrome or Gitelman syndrome. Jacq *et al.* described three patients with three different *CLCNKB* mutations whose clinical course was characterized by gradual transition from classic Barter syndrome to Gitelman syndrome phenotype [26]. A more recent study reported a large inbred Bedouin kindred from Northern Israel, which had 12 members who were affected with hypokalaemic tubulopathy [27]. The findings of the study demonstrated intrafamilial heterogeneity, namely the presence of Gitelman syndrome and classic Barter syndrome phenotypes in affected family members, all of whom were homozygous for a R438H mutation in the *CLCNKB* locus.

These studies provide evidence that Gitelman syndrome, previously considered a genetically homogenous disorder, can be caused by mutation in a gene other than *SLC12A3* (Table 1, Figure 1). The wide range of clinical phenotypes observed in patients with *CLCNKB* mutations has been attributed to alternative routes for basolateral Cl\textsuperscript{–} exit such as the KCl co-transporter, the cystic fibrosis transmembrane conductance regulator (CFTR) or the voltage-gated Cl\textsuperscript{–} channel, ClC5 [27]. It has been suggested that variation in expression and/or function in any one of these alternative transport systems may modify to variable degrees the derangement in ClC-Kb function, thereby influencing the disease phenotype [27]. Such a modifying effect could occur at the cellular/regulatory level, whereby one or more transport mechanisms are recruited to compensate for impaired ClC-Kb function or, alternatively, may be determined at the level of modifier genes.

A recent molecular finding, which has shed additional light on the molecular pathophysiology of Barter syndrome, was the identification by Hildebrandt’s and Jentsch’s groups of barttin, a β-subunit for ClC-Ka and ClC-Kb chloride channels (Table 1, Figure 1) [28,29]. Barttin is a protein encoded by the *BSND* gene, mutations in which cause...
the variant of antenatal Bartter syndrome with sensorineural deafness and renal failure (Table 1). Barttin co-localizes with the subunit of the Cl− channel in basolateral membranes of the renal tubule and inner ear epithelium [29]. It appears to mediate Cl− exit in the TAL (Figure 1) and Cl− recycling in K+‐secreting strial marginal cells in the inner ear.

Abnormalities in urinary mineral excretion

The loss-of-function mutations in the genes of TAL cell transporters in Bartter syndrome lead to the derangements in tubular handling of minerals observed in this syndrome [2,4,30]. Normally, the lumen to cell flux of Cl− via the NKCC2 co-transporter in the TAL and the exit of K+ from cell to lumen generate lumen-positive electrical potential, which, in turn, drives paracellular Ca2+ and Mg2+ transport from lumen to blood (Figure 1). Impaired function of NKCC2 or ROMK in antenatal Bartter syndrome results in reduction of intraluminal positive charge, which leads to hypercalciuria and nephrocalcinosis. The transport defect in the TAL should have resulted in inhibition of Mg2+ reabsorption and hypomagnesaemia. The absence of hypomagnesaemia in patients with antenatal Bartter syndrome has been explained by compensatory stimulation of Mg2+ reabsorption in the distal convoluted tubule induced by the high level of aldosterone, which is a characteristic of the syndrome [2,12].

Most recently, gain-of-function mutations of the gene for the calcium-sensing receptor (CaSR) causing autosomal dominant hypocalcaemia have been added to the expanding list of molecular defects resulting in Bartter syndrome (Table 1, Figure 1) [8,9]. In the kidney, the CaSR is expressed mainly in the basolateral membrane of the cortical TAL (Figure 1) [31]. Activation of CaSR by high concentration of extracellular Ca2+ or Mg2+ or by gain-of-function mutation triggers intracellular signalling events, including production of arachidonic acid and inhibition of adenylate cyclase [31]. Both actions result in inhibition of ROMK activity, which, in turn, leads to reduction in the lumen-positive electrical potential. This effect of CaSR explains why patients with activating mutations in this receptor may present with both hypercalciuric hypocalcaemia and renal wasting of NaCl, resulting in Bartter-like syndrome. This association further underscores the strong link between tubular handling of minerals and renal salt homeostasis.

The exact mechanisms underlying the hypercalciuria and hypomagnesaemia in Gitelman syndrome remain to be elucidated. It has been hypothesized that the loss-of-function mutation in TSC causes hypercalciuria by the same mechanism as thiazides (Figure 1) [4]. According to this hypothesis, impaired Na+ reabsorption across the luminal membrane of the distal convoluted tubular cell, coupled with continued exit of intracellular Cl− through basolateral Cl− channels, causes the cell to hyperpolarize. This, in turn, stimulates entry of Ca2+ into the cell via luminal voltage-activated Ca2+ channels [32]. In addition, the lowering of intracellular Na+ concentration facilitates Ca2+ exit via basolateral Na+/Ca2+ exchanger. The reasons for renal Mg2+ wasting and hypomagnesaemia typical of Gitelman syndrome are unknown [30].

Conclusion

In the past decade, remarkable progress has been made in our understanding of the molecular pathogenesis of Bartter syndrome. Molecular genetics and molecular biology studies have led to the identification of important Bartter syndrome-causing mutations, have provided important insight into the defective molecular mechanisms underlying this complex syndrome and have greatly increased our understanding of the physiology of transport processes in the TAL and the DCT. Nevertheless, several issues remain unsettled and warrant additional research. Further studies are needed to define defects in other transporter genes that may underlie specific forms of the disease, to investigate the phenotypic variability observed in specific genotypic variants and to explore the role of modifier genes in this variability. The exact role of specific mutations in the pathogenesis of the electrolyte and mineral abnormalities in Bartter syndrome and Gitelman syndrome is a subject for future research that may include site-directed mutagenesis and functional studies in heterologous expression systems, as well as the use of transgenic animals. These studies may significantly improve our understanding of the mechanisms underlying renal salt homeostasis, urinary mineral excretion and blood pressure regulation in health and disease.

Conflict of interest statement. None declared.

References


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Do protection devices have a role in renal angioplasty and stent placement?

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**Keywords:** renal angioplasty; renal atherosclerotic vascular disease; stent placement

In renal atherosclerotic vascular disease there is often no firm randomized trial data to guide clinical practice. Nephrologists often have to interpret trial data from other vascular territories. Vascular protection devices are now an issue in the carotid and cardiac territories. Should they have a role in renal atherosclerotic disease?

There is considerable literature on renal atheroembolic disease. The early paper of Thurlbeck and Castleman [1] showed that in post-mortem renal histology analysis after aortic aneurysm repair followed by death there was an incidence of renal atheroembolic