Molecular pathophysiology of Bartter's and Gitelman's syndromes

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Background: **In the last two decades, progress in cytogenetic and genome research has enabled investigators to unravel the underlying molecular mechanisms of inherited tubulopathies such as Bartter's and Gitelman's syndromes and helped physicians to better understand not only these two pathologic entities but also renal pathophysiology and salt sensitive hypertension.**

Data sources: **Articles collected from PubMed and open access journals included original articles, research articles, and comprehensive reviews. They were evaluated by the authors with an special emphasis on originality and up to date information about molecular pathophysiology.**

Results: **Bartter's and Gitelman's syndromes are two different inherited salt loosing tubulopathies. They are characterized by various inability of distal nephron to reabsorb sodium chloride with resultant extarcellular volume contraction and increased activity of the renin angiotensin aldosterone system. Hypokalemic metabolic alkalosis is a common feature of these two forms of tubulopathies. Hypercalciuria characterizes the majority of Bartter's syndrome, and hypomagnesemia with hypocalciuria characterizes Gitelman's syndrome. Low blood pressure is a common feature among patients who suffered from these tubulopathies. Bartter's syndromes encompass a heterogeneous group of ion channels defects localized at the thick ascending limp of Henle's loop with resultant loss of function of sodium-potassium-2 chloride cotransporter. These defects result in the impairment of the countercurrent multiplication system of the kidney as well as calcium, potassium and acid base disturbances which in the majority of cases are proved lethal especially in the antenatal and/or immediate postnatal life period. The underlying pathology in Gitelman's syndrome is defined to the distal convoluted tubule and is related to loss of function of the sodium-chloride cotransporter. The results of this defect encompass the inability of extracellular volume homeostasis, magnesium and potassium conservation, and acid base disturbances which are generally mild and in the majority of cases are not life-threatening.**

Conclusions: **Recent advances in molecular pathophysiology of Bartter's and Gitelman's syndromes have helped physicians to better understand the underlying mechanisms of these pathologic entities which remain obscure. Data collected from experiments among genetically manipulated animals enable us to better understand the pathophysiology of mammalian kidney and the underlying mechanisms of salt sensitive hypertension and to lay a foundation for the future development of new drugs, especially diuretics and antihypertensive drugs.**

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Key words: **Bartter's syndrome; calcium reabsorption; Gitelman's syndrome; magnesium reabsorption; salt loosing tubulopathies**

Review article

Introduction

In 1962 Bartter and his colleagues[1] reported two African American patients, a 5-year old boy and a 25-year old adult, with weakness, fatigue, slow growth, episodes of tetany, diarrhea and dehydration. Laboratory findings revealed the presence of hypokalemic, metabolic alkalosis with low levels of serum chloride and increased levels of aldosterone and angiotensin II (Ang II). Renal biopsy showed increased volume of the juxtaglomerular apparatus. By these two cases, they defined the presence of a new syndrome as Bartter's syndrome.

Four years later, in 1966, Gitelman and colleagues[2] described three young females, two of whom were sisters, with the presence of hypomagnesemia, hypokalemia and metabolic alkalosis with impaired renal conservation of potassium and magnesium. The patients exhibited high normal aldosterone levels and increased plasma renin activity. By these three cases,

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they described the presence of a new familial disorder as Gitelman's syndrome.

The overall incidence of Bartter's syndrome is estimated to be 1.2/million population, but the incidence of mutations affecting the solute carrier family 12 member 1 gene (*SLC12A1*) and potassium inwardly-rectifying channel, subfamily J, member 1 gene (*KCNJ1*), as estimated among participants of Framingham Heart Study, was found to be 1/360 and 1/670, respectively, indicating a frequency of about 1/100 000 population for Bartter's syndrome, which is much more greater than that observed in epidemiological studies. This discrepancy may be attributed to the fact that Bartter's syndrome is associated with an increased antenatal and neonatal mortality and many phenotypes do not thrive.[3,4] The prevalence of Gitelman's syndrome is estimated to be 1:40 000 in the general population and the prevalence of heterozygotes in the Caucasian population is approximately 1%.[5]

Patients with Bartter's syndrome, especially those with antenatal manifestation, exhibit increased urine levels of prostaglandin E2 (PGE2) and diminished susceptibility to the pressor effect of Ang II and norepinephrine. Early detailed investigation of renal function, mainly by free water clearance and solute clearance, showed that Bartter's syndrome represents a heterogeneous group of patients with more than one defect in distal nephrons.[6]

We recognize that Bartter's and Gitelman's syndromes represent two different groups of inherited salt loosing tubulopathies characterized by hypokalemic metabolic alkalosis and normal or low blood pressure with increased renin activity and high aldosterone levels.[7] These two syndromes share many common clinical and laboratory findings but can be distinguished upon special clinical and laboratory findings that characterize each other. Bartter's syndrome appears early in the life often with troublesome symptoms such as tetany, vomiting, hypokalemia and dehydration which constitute life-threatening situations and need prompt therapy. It is characterized by the presence of polyuria, polydipsia, hypercalciuria, and frequently nephrocalcinosis.[7,8] Gitelman's syndrome appears later in the life with mild symptoms, mainly muscle cramps or spasms, which are easily controlled by increased consumption of salt and potassium containing foods. It is characterized by the presence of hypomagnesemia and hypocalciuria.[7,8]

The underlying pathophysiological abnormality is the inability of the distal nephron to manipulate effectively sodium chloride reabsorption with resultant extra cellular volume contraction and increased activity of the renin-angiotensin-aldosterone system (RAAS). In Bartter's syndrome, the defective mechanism is located in the thick ascending limb (TAL) of Henle's loop and comprises loss of function of the sodium/potassium/2 chloride cotransporter-2 (NKCC2) or a group of other proteins which lead to secondary loss of function of NKCC2. In Gitelman's syndrome, the defective mechanism is located in the distal convoluted tubule and comprises loss of function of the sodium-chloride cotransporter (NCC).[7,8]

It is well known that NKCC2 is the target of loop diuretics such as bumetanide and furosemide, and NCC is the target of thiazide diuretics. Clinical and laboratory findings among patients with Bartter's syndrome resemble those of chronic abuse of loop diuretics and among patients with Gitelman's syndrome resemble those of chronic abuse of thiazide diuretics. Before invention of genomic analysis and genetic confirmation of these two pathologic entities, it was possible to distinguish each other by administration of loop and/or thiazide diuretics and investigate their effects upon renal function.[8,9]

In the early 1990s, Gamba et al[10,11] cloned and purified, from the urinary bladder of the fish winder flounder, the proteins which constitute the NCC and NKCC2. A few years later, Simon et al[12-14] reported that mutations in the gene encoding the NCC protein are responsible for the presence of Gitelman's syndrome and mutations in the gene encoding the NKCC2 protein are responsible for Bartter's syndrome type I. They also found that mutations in the gene encoding renal outer medullary potasium (ROMK) channel protein are responsible for Bartter's type II.

Loss of function of NKCC2 may be due to inactivating mutations of the gene encoding the protein itself as well as mutations involving the genes of other proteins which play a crucial role upon the function of NKCC2. ROMK channel, chloride channel Kb (ClC-Kb), Bartin and calcium sensing receptor (CaSR) also produce a Bartter's like syndrome although there is a different inheritance mode.[7,15]

Genetic analysis among members from suffered families unraveled the mystery of Bartter's like syndrome and opened a new window for understanding renal physiology as well as inherited forms of sodium sensitive hypertension.

Management of patients with Bartter's and Gitelman's syndromes is oriented toward acute and chronic therapy of presenting complications and abnormalities. Patients with Bartter's syndrome are more prone to life-threatening complications, especially during the postnatal period, such as volume depletion, diarrhea, spasm, fever and dangerous hypokalemia. Prompt therapy with volume and electrolyte restoration is needed in order to save patient's life.[4,7]

Chronic therapy of underlying abnormalities such as increased prostaglandins synthesis and RAAS activity, which aggravate electrolyte and acid base disturbances,

includes potassium supplementation and application of cyclooxygenase inhibitors, angiotensin converting enzyme (ACE)-inhibitors and potassium sparing diuretics.[7] Indomethacin has been used extensively in the treatment of Bartter's syndrome at a dose of 2-3 mg/kg per day (mean 2.1 mg/kg per day) and proved efficient in increasing height, body weight and reducing hyperfiltration.[7,16]

Nevertheless, indomethacin administration is coupled with frequent gastrointestinal complications such as gastritis, bleeding ulcers and necrotizing enterocolitis especially in infants. Proteinuria may be another complication of indomethacin administration. Use of newer selective cyclooxygenase (COX)-2 inhibitors such as celecoxib is coupled with less frequent gastrointestinal complications and is better tolerated but there are some concerns about its potential adverse cardiovascular effects. When proteinuria complicates COX inhibitors, substitution of the drugs with ACE-inhibitors such as enalapril is recommended but special caution should be taken because of the possibility of serious hypotensive episodes.[7,16]

Chronic treatment of patients with Gitelman's syndrome comprises oral potassium and magnesium supplementation with adequate salt and water consumption in order to maintain effective extracellular volume. Indomethacin, amiloride and eplerenone have been used to treat hypokalemia. Indomethacin is more efficacious than the other two agents but produces more frequently gastrointestinal complications. Hence it is recommended to evaluate the risk/benefit for individual patients while making a decision for the use of these drugs.[17]

Ion transport in the TAL of Henle's loop and distal convoluted tubule (DCT)

Under normal conditions, the TAL of Henle's loop reabsorbs about 25%-30% of filtrate in Bowman's capsule and the DCT, about 5%-10% of filtrate. These two parts of nephron possess an important role in extracellular volume and electrolyte conservation. Although the amount of filtrate reabsorbed in the DCT is only about 10% of the initial filtrate in Bowman's capsule, its topography beyond the macula densa and hence out of the regulatory mechanism of the tubulo-glomerular feedback disposes to this segment of nephron, a special role in fine tuning of volume and electrolyte regulation.[18]

The TAL is impermeable to water but it has the ability to reabsorb a large amount of sodium chloride which is transferred to the interstitium of the inner medulla and contributes to the countercurrent multiplication system which is responsible for urine concentrating ability in the mammalian kidney. Sodium chloride reabsorption in the TAL is considered to be the "single effect" of the countercurrent multiplication system in the mammalian kidney. Moreover, the reabsorption of sodium chloride in the TAL (diluting segment) produces a gradual dilution of urine so that urine reaching the distal convoluted tubule exhibits an osmolality equal to 150-200 mOsm/kg H_2O compared with 1200 mOsm/kg H_2O at the end of the loop of Henle in the deep medulla. The final concentration of urine takes place in the medullary portion of the collecting tubule as it passes through the hypertonic medulla. The DCT has the capacity to reabsorb sodium chloride as well as calcium, magnesium and water. It is also capable of reabsorbing sodium in exchange with potassium under the influence of aldosterone (Fig. 1).[18,19]

The main ion channel at the luminal surface of the TAL is the NKCC2 which belongs to the solute carrier family SLC12A of chloride channels.[11] It utilizes the energy produced by the Na^+-K^+-ATPase located at the basolateral membrane of epithelial cells and carries sodium-potassium and chloride inside the cell. The sodium is moved from the tubular lumen to the cell interior, while reducing its concentration and electrochemical gradient coupled by the two other

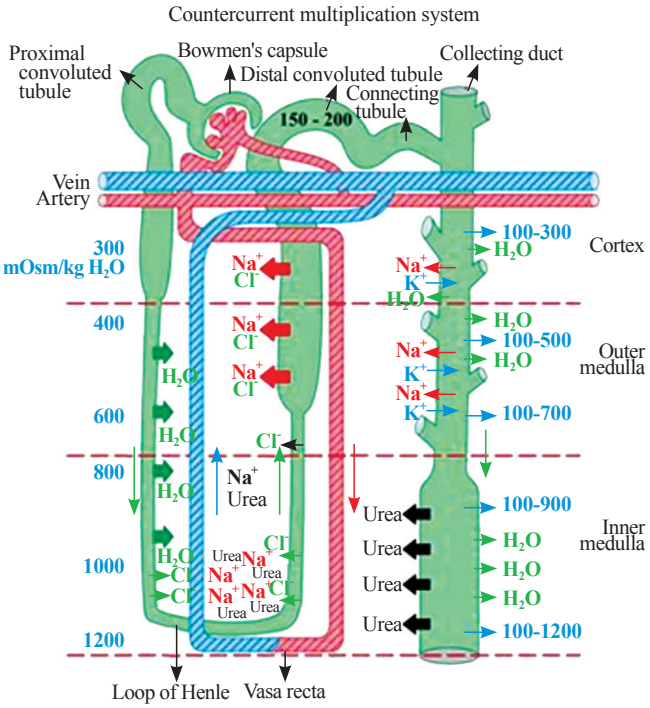

Fig. 1. The countercurrent multiplication system of the mammalian kidney is responsible for sodium and water conservation. The main functional units of the system are the Henle's loop, vasa recta and the collecting duct. Active sodium transport in the thick ascending limb is mainly responsible for inner medulla hypertonicity which is augmented via urea recycling from the IMCD. Water reabsorption is mainly accomplished at the level of IMCD under the permissive action of anti-diuretic hormone. IMCD: inner medullary collecting duct. (This figure was hand made and processed by a professional graphic designer.)

Review article

ions (potassium and chloride) in a stoichiometry of $1Na^+:1K^+:2Cl^-$, which ensures its electro neutrality. As shown by Greger and his colleague,[20] the presence of potassium and chloride is obligatory for the proper function of the transporter. Another important role of the TAL is the reabsorption of divalent cations such as calcium and magnesium which are reabsorbed by a passive paracellular mechanism.[18]

NKCC2 is the product of the *SLC12A1* gene located in chromosome 15q21.1 in humans. It has a molecular weight of about 121 kDa and is composed of 1095 amino acids. The protein is located at the luminal surface of the TAL and the macula densa cells sense the chloride concentration in the tubular lumen and regulate the tubulo-glomerular feedback. The membrane topology of the protein exhibits 12 hydrophobic transmembrane domains with a long hydrophilic loop between the 7th and 8th domains, and the amino and carboxy termini are located inside the cell membrane. The hydrophilic loop carries two glycosylation sites which are not entirely necessary for its functional activity.[11,21]

The functional activity of the protein is increased by hormones which increase the intracellular concentration of cyclic adenosine monophosphate such as vasopressin, parathyroid hormone (PTH), calcitonin, glucagon and β-adrenergic agonists. Conversely, its functional activity is diminished by hormones which increase intracellular cyclic guanosine monophosphate such as atrial natriuretic peptide, endothelin, α-adrenergic agonists and nitric oxide.[21]

The movement of sodium, potassium and chloride ions inside the cell alters the intracellular ion balance and hence they have to be removed either to the interstitium or to the tubular lumen. The sodium is removed to the interstitium by the function of Na^+-K^+-ATPase. Potassium is extruded to the tubular lumen by the ROMK channel located at the luminal surface of the epithelial cell and is the product of the *KCNJ1* gene. Part of the recycled potassium in the tubular lumen is utilized by the NKCC2 ensuing its proper function, and the remainder produces the intraluminal positive electrical charge (+5 to +10 mV) which is necessary for the paracellular reabsorption of the positive ions such as sodium, calcium and magnesium (Fig. 2).[21,22]

Chloride ions are removed to the interstitium either by the potassium-chloride cotransporter (KCC) or by the ClC-Kb located at the basolateral membrane of the cell. The ClC-Kb channel is the product of the chloride channel, voltage-sensitive Kb gene (*CLCNKB*). Effective function of ClC-Kb is ensured by the presence of a small protein (about 40 kDa) the beta subunit named Barttin which is a necessary accessory protein for ClC-Kb and ClC-Ka. Barttin, which is the product of the Bartin CLCNK-type chloride channel accessory beta subunit (*BSND*) gene, acts not only as a "chaperon" facilitating the trafficking of the two chloride channels to the plasma membrane but also affects their ion conductance, and its presence is necessary to make these two channels functional. The ClC-Ka is predominantly expressed in the thin limb of Henle's loop and the ClC-Kb in the TAL of Henle's loop, the DCT and in collecting duct intercalated cells. These two proteins with the Barttin subunit are also expressed in the inner ear and contribute significantly to the production of endolymph (Fig. 2).[23,24]

The lining epithelium of the initial part of the DCT, immediately after the macula densa, is identical to the TAL and is characterized by the expression of NKCC2, after that begins the main DCT followed by the connecting tubule (CNT). The DCT possesses, at its tubular membrane, two main sodium conducting channels, the NCC and the epithelial sodium channel (ENaC). Potassium conducting ROMK channels are also expressed at the luminal surface. In humans, the NCC is expressed at the initial 30% of the length of the DCT, after that NCC and ENaC are co-expressed in a short length of the tubule and at the end of the DCT only ENaC is expressed.[25]

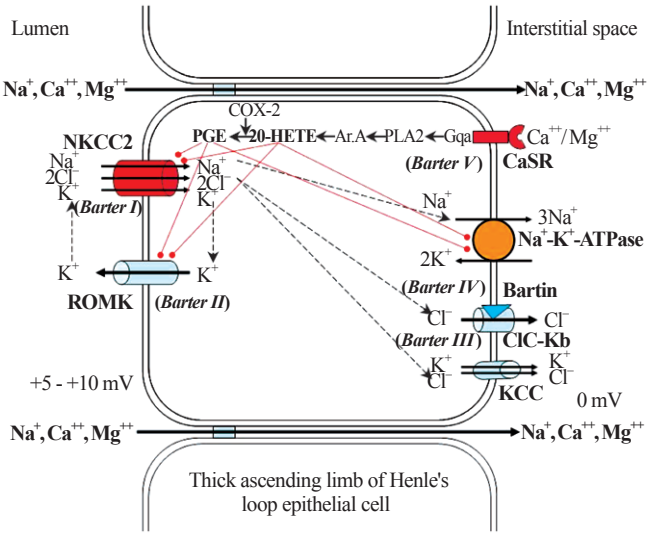

Fig. 2. Epithelial cells in the thick ascending limp of Henle's loop reabsorb about 25% of filtrate in Bowman's capsule. Active sodium reabsorption is accomplished via the NKCC2. About 20% of filtered calcium and 65%-70% of filtered magnesium are reabsorbed in this nephron segment via a passive paracellular way. Potassium is recycled to the tubular lumen via the ROMK channel. Chloride exits to the interstitial space via the KCC or the ClC-Kb. Bartin β-subunit is an essential accessory protein for ClC-Kb activation. CaSR is activated via the interstitial calcium and magnesium concentration and inactivates NKCC2, ROMK channel and Na^+-K^+-ATPase. NKCC2: sodium/potassium/2 chloride cotransporter-2; ROMK: renal outer medullary potassium channel; ClC-Kb: chloride channel Kb; CaSR: calcium sensing receptor; Gqa: G-protein qa; PLA2: phospholipase A2; Ar.A: arachidonic acid; 20-HETE: 20-hydroxyeicosatetranoic acid; PGE: prostaglandin E; COX-2: cyclooxygenase-2; KCC: potassium/chloride cotransporter. (This figure was hand made and processed by a professional graphic designer.)

The NCC is inhibited by thiazide diuretics and the ENaC by amiloride diuretics. The expression and functional capacity of ENaC are modulated by aldosterone and the inward moving sodium is counterbalanced by the excretion to the tubular lumen of potassium via the ROMK channel (Fig. 3).[26]

The NCC belongs to the solute carrier family SLC12A and it is the product of the solute carrier family 12 member 3 gene (*SLC12A3*) located in chromosome 16q13 in humans. The NCC protein is formed from 1002 to 1023 amino acids and its topology in cell membrane is identical to NKCC2 and exhibits 12 hydrophobic transmembrane domains and a long hydrophilic loop between the 7th and 8th transmembrane domains. The amino and carboxy-termini of the protein are oriented toward inside the cell membrane. The hydrophilic loop contains two glycosylation sites which are necessary for its proper function. Elimination of one glycosylation site diminishes NCC activity by 50%, and elimination of both sites reduces its activity by 95%.[10,26]

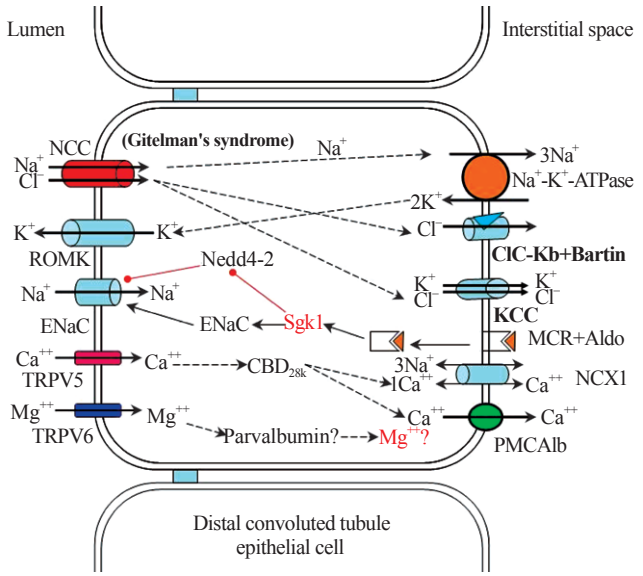

Fig. 3. Distal convoluted tubule epithelial cell reabsorb about 5%-10% of the initial filtrate in Bowman's capsule. The main sodium conducting channels are the NCC and the ENaC. NCC inactivating mutations are responsible for Gitelman's syndrome. ENaC is activated by aldosterone, sodium reabsorption is coupled with potassium excretion to the tubular lumen via ROMK channel. Chloride is transported to the interstitial space via KCC and ClC-Kb channels located at the basolateral membrane. Calcium and magnesium are reabsorbed via an active transcellular way. Calcium is reabsorbed via the TRPV5 channel and magnesium via the TRPM6 channel. NCC: sodium/chloride cotransporter; ENaC: epithelial sodium channel; ROMK: renal outer medullary potassium channel; KCC: potassium/chloride cotransporter; ClC-Kb: chloride channel Kb; TRPV5: transient receptor potential vanilloid-5; TRPM6: transient receptor potential melastin-6; CBD_{28k}: calbidin D-28k; Sgk1: serum/glucocorticoid regulated kinase 1; Nedd4-2: neutral precursor cell expressed developmentally down-regulated protein 4-2; MCR+Aldo: mineral corticoid receptor+aldosterone; PMCA1b: plasma membrane Ca^{2+}-ATPase; NCX1: Na^+/Ca^{2+} exchanger. (This figure was hand made and processed by a professional graphic designer.)

The binding site of thiazide diuretics is not exactly known, but it is probably located between the transmembrane domains 8-12. NCC is also inhibited by a natural inhibitor synthesized by the epithelial cell and belongs to the family of serine/threonine kinases known as with no lysine kinases (WNKs) and especially the WNK4 which inhibits also the function of ENaC and ROMK channels.[26]

The calcium and magnesium absorption in the DCT is accomplished via an active transcellular process. For a long time, the transepithelial mechanism(s) of calcium and magnesium transport was elusive until it became evident that certain members of the transient receptor potential (TRP) channels were responsible for transmembrane calcium and magnesium absorption in the intestine and kidney epithelium. Two members of TRP channels such as the transient receptor potential vanilloid-5 (TRPV5) and the transient receptor potential melastin-6 (TRPM6) are expressed in distal nephron and are responsible for reabsorption of transepithelial calcium and magnesium, respectively.[27]

The TRPV5 is expressed predominantly in the lumen surface of DCT and CNT epithelial cells and is responsible for transmembrane transport of calcium, and it is accompanied by the calcium binding protein calbidin-D_{28K}, which acts simultaneously as a calcium binding and storage protein and is responsible for trafficking calcium intracellularly to the basolateral membrane where calcium is transferred to the extarcellular space via the calcium/sodium exchanger (CNX1) and the plasma membrane Ca^{2+}-ATPase (PMCA1b) (Fig. 3).[15,27]

The TRPM6 is expressed at the luminal surface of DCT cells and is responsible for transepithelial

Table. Classification of Bartter's and Gitelman's syndromes

Types	Channel	Genes	Chromosome	Heredity
Bartter I	NKCC2	*SLC12A1*	15q21.1	Autosomal recessive
Bartter II	ROMK	*KCNJ1*	11q24.3	Autosomal recessive
Bartter III (classic Bartter)	ClC-Kb	*CLCNKB*	1p36.13	Autosomal recessive
Bartter IV	Bartin	*BSND* (*CLCNKA*/ *CLCNKB*)	1p (1q32.3)	Autosomal recessive
Bartter V	CaSR	*CASR*	3q13	Autosomal dominant
Gitelman	NCC	*SLC12A3*	16q13	Autosomal recessive

NKCC2: sodium/potassium/2 chloride cotransporter-2; ROMK: renal outer medullary potassium; ClC-Kb:chloride channel Kb; CaSR: calcium sensing receptor; NCC: sodium/chloride cotransporter; *SLC12A1*: solute carrier family 12 member 1 gene; *KCNJ1*: potassium inwardly-rectifying channel, subfamily J, member 1 gene; *CLCNKA*: chloride-channel, voltage-sensitive Ka gene; *CLCNKB*: chloride-channel, voltage-sensitive Kb gene; *BSND*: Bartin CLCNK-type chloride channel accessory beta subunit gene; *CASR*: calcium-sensing receptor gene; *SLC12A3*: solute carrier family 12 member 3 gene.

transport of magnesium in this segment of nephron. The cellular trafficking and basolateral membrane transport of magnesium are not yet entirely known although parvalbumin is recently considered to be a suitable candidate as an intracellular magnesium buffer and a trafficking protein.[15,27,28]

Bartter's syndrome

Bartter's syndromes are rare hereditary disorders which encompass a heterogeneous group of salt loosing tubulopathies confined at the level of TAL with diverse underlying causes but with a common end-point pathophysiology characterized by loss of function of NKCC2.

The most frequent mutations encountered among Bartter's syndrome phenotypes are those affecting the *KCNJ1* gene (ROMK channel) followed by the *SLC12A1* gene (NKCC2 channel), *CLCNKB* gene (ClC-Kb channel), and *BSND* gene (Barttin protein). Nephrocalcinosis is a constant finding among phenotypes with *KCNJ1* and *SLC12A1* gene mutations. Chronic renal failure, although rare, is encountered among phenotypes with *KCNJ1*, *CLCNKB* and *BSND* gene mutations without the obligatory presence of nephrocalcinosis. Hearing loss is exclusively found among patients with *BSND* gene mutations (Fig. 2).[29]

Seyberth[30] has proposed a new terminology and classification of Bartter-like syndromes based upon the site of tubular dysfunction and divides them into three major types. Distal convoluted (DC) type is referred to distal convoluted tubule dysfunction caused by loss of function of NCC or ClC-Kb channels producing a mild form of salt losing tubulopathy relevant to Gitelman's syndrome or classic Bartter's syndrome type III. L type is referred to loop dysfunction caused by loss of function of NKCC2 or ROMK channels and represents a more severe form of salt loosing tubulopathy relevant to Bartter type I and type II syndromes. The L-DC type is a mix category of loop and distal tubule dysfunction caused by loss of function of ClC-Ka and ClC-Kb or the β-subunit Bartin. This type represents a severe form of salt loosing tubulopathy relevant to Bartter type IV syndrome.

Although Seyberth's classification is more easy and relevant to current knowledge upon salt loosing tubulopathies, the literature has not widely accepted this terminology and for simple reasons we shall approach Bartter's syndrome according to affected channels and we shall divide them into five subtypes (Table).

Bartter type I

The underlying cause is loss of function of NKCC2 as a result of inactivating mutations affecting the *SLC12A1* gene located in chromosome 15q21.1 and is inherited by the autosomal recessive mode. It is also known as prenatal Bartter or hyper prostaglandin E syndrome (HPS). Prenatal diagnosis of Bartter type I syndrome is suspected when unexplained polyhydramnios is present between 24 and 36 gestational weeks. Polyhydramnios is produced by increased urinary output from the fetus as a result of decreased urinary concentrating ability and polyuria and leads to premature birth. Increased chloride level of amniotic fluid is also a hallmark of prenatal diagnosis. The most striking clinical symptom in neonatal life is an excessive polyuria.[13,29,31]

Increased PGE2 level in the blood and urine of affected patients is a constant finding. The most probable explanation for increased PGE2 synthesis is the fact that the macula densa cells sense the intraluminal chloride concentration and modulate accordingly the production of prostaglandins. NKCC2 is the predominant chloride channel in macula densa cells which by increasing intracellular chloride concentration decreases the activity of COX-2 while reducing prostaglandin synthesis. The RAAS system is regulated via prostaglandin synthesis by the macula densa cells. Loss of function of NKCC2 is coupled with low intracellular chloride transport which produces a false sensation of low chloride concentration in the tubular fluid of TAL and increases the activity of COX-2 that increases the production of PGE2 and the activity of the RAAS system.[32]

Another important finding among these patients is the increased levels of renin, Ang II and aldosterone. The increased level of these hormones is the result of increased PGE2 production and the response to extarcellular volume contraction as a result of impaired urine concentrating ability and salt loosing from distal nephron.[29,31,32]

Patients with Bartter type I exhibit plasma renin levels 13 (10-31) times above the upper limit for age and aldosterone levels approximately 3 times above the upper limit for age. Increased plasma renin activity levels are more constantly found among these patients than aldosterone levels.[29,33]

Reduced plasma potassium and chloride levels are frequent but not invariably constant findings among these patients. The same is true for plasma bicarbonate levels which are mildly increased in the majority of patients.[29,33]

Hypokalemia is the result of increased aldosterone level which increases the activity of ENaC in DCT resulting in increased sodium reabsorption and potassium excretion via ROMK channel.[34] Another cause of hypokalemia is the increased potassium excretion in DCT via the big potassium (BK) channels as a result of increased solute delivery to the distal nephron because BK channels activity is flow

dependent.[35] Reduced chloride reabsorption in TAL is partially restored by increased sodium chloride reabsorption in the DCT by NCC cotransporter and so hypochloremia is not a profound feature.[34]

Urine loss of hydrogen ions and mildly increased reabsorption of bicarbonate ions with resultant metabolic alkalosis is the result of increased aldosterone levels and reduced potassium levels. Aldosterone increases vacuolar ATPase (V-ATPase) activity in the luminal surface of α-intercalated cells and chloride/bicarbonate exchanger (AE1) in the basolateral membrane of these cells. The substrates of these two ion transporters (hydrogen and bicarbonate) are formed intracellularly from CO_2 and water under the influence of carbonic anhydrase II. Combination of these actions results in proton excretion to the tubular lumen and bicarbonate transport to the interstitium in exchange with chloride anions to the intracellular space via the AE1.[34,36]

Hypercalciuria is a constant finding among these patients and medullary nephrocalcinosis develops during the first month of life.[29,34] Hypercalciuria is the result of NKCC2 defective function which increases intraluminal concentration of chloride ions and produces negative intraluminal charge. The negative intraluminal charge prevents the paracellular reabsorption of positive ions such as calcium. Furthermore, increased delivery of sodium chloride to the DCT increases the activity of NCC cotransporter which is coupled with diminished calcium transcellular reabsorption and further aggravates hypercalciuria.[34]

Magnesium conservation is a mystery among these patients because loss of intraluminal positive charge of the TAL affects paracellular reabsorption of calcium as well as magnesium but hypomagnesemia is a very rare finding among these patients except a substantial percentage of patients affected by *CLCNKB* mutations responsible for classic (type III) Bartter's syndrome. Since 65%-70% of filtered magnesium is reabsorbed in the TAL, it is possible that increased magnesium reabsorption in the DCT compensates for diminished magnesium reabsorption in the TAL but the underlying mechanism(s) for such a compensation is still unknown.[34,37]

Bartter type II

The underlying cause is loss of function mutations affecting the *KCNJ1* gene located in chromosome 11q24.3 and is inherited by the autosomal recessive mode. It is also known as neonatal or in most cases prenatal Bartter's syndrome with transient hyperkalemia or HPS. Mutations affecting the *KCNJ1* gene are the most frequent among patients with Bartter's syndrome and all known mutations are located in exon 5.[7,29,33]

The underlying pathophysiology of Bartter type II is attributed to the fact that the defective function of ROMK channel leads to disruption of potassium recycling from the intracellular compartment to the tubular lumen of the TAL. The presence of potassium and chloride in the tubular lumen is obligatory for the proper function of NKCC2, hence the ROMK channel is a powerful regulator of NKCC2 functional activity and its defective function leads to NKCC2 inactivation.[14,20]

Clinical presentation is almost identical to Bartter type I with the exception of transient hyperkalemia during the first few days of life associated with normal acid-base status or either metabolic acidosis. Hyperkalemia is the result of defective ROMK function but gradually the BK potassium channels of the DCT increases potassium excretion in distal nephron and serum potassium levels fall. Aldosterone hyperactivity increases sodium reabsorption via ENaC and hydrogen excretion via V-ATPase with resultant metabolic alkalosis but generally patients with Bartter type II exhibit normal or mildly increased serum potassium levels compared with patients with other Bartter's syndrome.[29,33]

Polyhydramnios and premature birth are also present among these patients. Hypercalciuria is present and leads to nephrocalcinosis during the first two months of life. Mildly reduced chloride levels and mildly increased bicarbonate levels are encountered. Aldosterone levels are approximately 3.5 times greater than the upper normal limit for age and renin levels are approximately 6 times greater than the upper normal limit for age, but mean renin levels are the lowest in patients with Bartter's syndrome.[29,33]

Bartter type III

The underlying cause is loss of function mutations affecting the *CLCNKB* gene located in chromosome 1p36.13 and is inherited by the autosomal recessive mode. The encoding protein forms the ClC-Kb chloride channel located at the basolateral membrane of the TAL and DCT where its expression is more abundant. It is known as classic or postnatal Barter's syndrome, although there are cases with antenatal presentation, and is the third most frequent abnormality encountered among patients with Bartter's syndrome.[29,38]

Since the original confirmation of genetic linkage between *CLCNKB* mutations and Bartter type III phenotype, by Simon et al[38] and three years later by Konrad et al,[39] it became evident that the clinical presentation of these patients is characterized by a considerable diversity from life-threatening dehydration and hypotension during the first year of life to polyuria and/or dehydration diagnosed later in life as well as hypocalciuria and hypomagnesemia resembling Gitelman's syndrome.

The reason for this phenotypic diversity is not exactly known. We know that loss of function of ClC-Kb is coupled with severe impairment of chloride reabsorption in the TAL. Under normal circumstances, the fractional chloride reabsorption in the TAL is estimated to be 80%-95%. Conversely, patients with Bartter type III exhibit fractional chloride reabsorption 24%-25% as estimated by Simon et al.[38] The phenotypic diversity implies that chloride movement through the basolateral membrane to the interstitium is accomplished via alternative pathways such as KCl cotransporter and ClC-Ka which may partially compensate for intracellular chloride accumulation and result in partial NKCC2 dysfunction.[29,39]

ClC-Ka is primarily expressed in the thin limp of Henle's loop, especially in the medullary portion, where it plays an important role in chloride reabsorption and contributes to the medullary hypertonicity, but it is also expressed in the TAL, DCT and cortical collecting duct. Its expression is up-regulated by cell volume contraction and loss of function of NKCC2. The ClC-Ka as well as ClC-Kb needs Barttin β-subunit in order to be fully activated. Barttin is up-regulated by the protein kinase-serum/glucocorticoid regulated kinase 1 (SgK1) which increases after aldosterone coupling with its receptor, but it is also known that the *SGK1* gene is expressed under circumstances of cell volume contraction with resultant cell shrinkage. These mechanisms are fully activated in Barter's syndrome and may be responsible for alternative chloride transport from the intracellular compartment to the interstitium, as a result of CLC-Ka hyperactivity, and hence partial restoration of NKCC2 activity.[40]

The resemblance of some patients with Gitelman's syndrome is explained by the fact that ClC-Kb is expressed in much more abundance in the DCT than in the TAL and hence channel dysfunction is coupled with partial loss of function of NCC in this nephron segment. Jeck et al[41] described three patients with mixed Bartter-Gitelman phenotype with hypomagnesemia and hypocalciuria, but genomic analysis showed mutations in the *CLCNKB* gene.

Bartter type III patients are characterized by the inability of chloride conservation and they exhibit the lowest serum chloride levels than any other patient affected by Bartter's syndrome. They also exhibit invariably increased levels of serum bicarbonate and low levels of potassium. The levels of renin and aldosterone are modestly elevated. Nephrocalcinosis is not present in early life and manifests during childhood or adolescent, with a median age of 15.5 years.[29]

Bartter type IV

The underlying cause is loss of function mutations affecting the *BSND* gene encoding the Barttin β-subunit which is located in chromosome 1q32.3 and is a necessary accessory protein for the proper function of both chloride channels ClC-Ka and ClC-Kb.[42]

Bartter type IV is also known as antenatal Bartter's syndrome with sensorineural deafness or HPS and inherited by the autosomal recessive mode. It represents the most severe form of disease characterized by excessive polyhydramnios and prematurity with early failure to thrive. Although nephrocalcinosis is almost absent, most patients exhibit renal failure owing to renal tissue damage such as glomerular sclerosis, tubular atrophy and mononuclear cell infiltration which usually leads to renal failure.[29,34]

The severity of Bartter type IV is explained by the fact that Barttin loss of function leads to inactivation of both chloride channels ClC-Ka and ClC-Kb while abolishing chloride transport through the basolateral membrane of the thin ascending limp of Henle's loop, the TAL and DCT which affect severely the concentrating ability of the kidney as well as the capacity of salt reabsorption in distal nephron.[34,40]

Calcium homeostasis is conserved, but magnesium loss and hypomagnesemia are progressively established. Lack of hypercalciuria may be explained by the fact that diminished paracellular calcium reabsorption in the TAL is counterbalanced by increased transcellular calcium reabsorption in other nephron segments as it is encountered during thiazide diuretic administration. For the same reason, magnesium loss in the TAL is continued in the DCT because of NCC dysfunction as it is encountered in Gitelman's syndrome (see forward).[34]

Sensorineural deafness is the result of ClC-Ka and ClC-Kb inactivation in the stria vascularis and the vestibular dark cells of the inner ear. It is known that both chloride channels co localizes with Barttin in order to form functional heterodimers and are expressed in the inner ear and contribute significantly to endolymph formation. Although endolymph is of extarcellular origin, its ion composition resembles to intracellular fluid and hence potassium ions concentration predominates over sodium ions concentration.[43]

Marginal cells are capable of transferring potassium ions from capillary circulation to endolymph via the following mechanism. The sodium/potassium/2 chloride cotransporter-1 (NKCC1) is the main cotransporter among these cells and is located at the basolateral membrane of the marginal cells and transfer sodium, potassium and chloride into the intracellular compartment. Sodium is pumped out to the interstitial fluid via Na^+-K^+-ATPase, chloride exits to the interstitium via the ClC-Ka/Barttin and ClC-Kb/Bartin heterodimers located also at the basolateral membrane, and potassium is transferred to endolymph via the potassium channels (potassium voltage-gated channel, KTQ-like subfamily, member 1/potassium voltage-gated

channel, Isk-related family, member 1) located at the apical membrane of the cell, similar to ROMK channels. Recycling of chloride from the intracellular to interstitial space is necessary for the proper function of NKCC1 and potassium transfer to endolymph. Disruption of this machinery leads to reduced potassium exit from the apical cell membrane and hence reduced potassium content of endolymph which leads to hearing loss.[43]

Bartter type IV is also encountered among patients suffered from digenic mutations affecting concomitantly the *CLCNKA* and *CLCNKB* genes which results in loss of function of ClC-Ka and ClC-Kb chloride channels. Both genes are located closely at chromosome 1p36 and in extremely rare occasions mutations affecting both genes with Bartter type IV phenotype have described. These cases represent an atypical pattern of inheritance known as digenic inheritance which has been the subject of intensive investigation in the past decade and showed that other diseases such as retinitis pigmentosa, Bardet-Biedl syndrome, deafness, cortisone reductase deficiency, early onset of Parkinson disease and early onset of autosomal dominant polycystic kidney disease can be inherited by the same mode.[44,45]

Bartter type V

It is also known as hypocalcemia with Bartter like syndrome. The underlying cause is gain of function mutations of the CaSR encoded by the gene *CASR* located at chromosome 3q13 and is inherited by the autosomal dominant mode.[7,46]

The initial phenotype of these patients is that of autosomal dominant hypocalcemia (ADH) with tetany and low levels of PTH. Later during their life expectancy hypokalemia, hypomagnesemia, metabolic alkalosis, hyper-reninaemia and hyper-aldosteronaemia are superimposed to the clinical picture pointing towards Bartter's syndrome. The mixed phenotype between ADH and Bartter's syndrome prompted the investigators to seek possible mutations affecting the *CASR* gene.[47,48]

The first description reported one patient with the previous phenotype and revealed a mutation affecting CaSR with substitution of leucine for proline in the position 125 (L125P) which is coupled with gain of function of CaSR.[47] The second description reported two patients and revealed in the first patient a mutation at codon 843 resulting in substitution of adenine for cytosine (A843E) and in the second patient a mutation at codon 131 resulting in substitution of cysteine to tryptophan (C131W) which showed also activating mutations of *CaSR*.[48]

The CaSR belongs to the C family of G-protein coupled receptors and plays an important role in calcium homeostasis. The receptor is primarily expressed in parathyroid gland cells and the kidney. In the parathyroid gland regulates PTH secretion, PTH synthesis and parathyroid cellular proliferation. Activation of the receptor suppresses the production of PTH and the proliferation of parathyroid cells; conversely inactivation of the receptor increases the secretion of PTH and produces parathyroid hyperplasia although in the presence of hypercalcemia.[47]

In the kidney, CaSR is expressed throughout the nephron although not in the same membrane polarization and density. In the cortical TAL, the receptor is expressed at the basolateral membrane and its activation by increased concentration of extracellular calcium and/or magnesium inhibits sodium chloride and divalent cations (calcium and magnesium) reabsorption by complex molecular pathways which are not fully understood until now.[46]

Activation of basolateral membrane CaSR in the TAL leads to inactivation of NKCC2, ROMK and Na^+-K^+-ATPase and causes decreased reabsorption of calcium and magnesium in such a manner as the extarcellular concentration of these anions is capable of regulating their own handling by the kidney. The underlying molecular mechanism of this regulation has been extensively studied and is attributed to intracellular molecular actions of the receptor involving activation of phospholipase A2 (PLA2) via G-protein qa (Gqa).[49] Activation of PLA2, by increased concentration of intracellular calcium, leads to increased arachidonic acid production which in turn is rapidly metabolized to 20-hydroxyeicosatetraenoic acid (20-HETE) and in advance to prostaglandins via COX-2 activation. 20-HETE and prostaglandins inhibit NKCC2, ROMK and Na^+-K^+-ATPase activity, leading to diminished sodium chloride, calcium and magnesium reabsorption by the TAL (Fig. 2).[49]

Another mechanism by which CaSR affects electrolyte reabsorption by the TAL is the capacity of the receptor to inhibit the activity of basolateral inwardly rectifying potassium channels (Kirs) such as Kir4.1 and Kir5.1.[50,51] The inhibition of these channels is mediated by activation of phospholipase C and protein kinase C pathways via activation of Gqa. The Kirs channels at basolateral membrane transport potassium from the intracellular space to the interstitial space increase the negative electrochemical gradient of cell membrane which is necessary for intracellular chloride transport to the interstitium. Hence, Kirs inactivation leads to intracellular chloride accumulation.[51]

Intracellular chloride accumulation leads to NKCC2 inactivation via a complicated mechanism which includes WNKs and Ste20-related proline alanine rich kinase (SPAK)/oxidative stress responsive 1 kinase (OSR1). It is known that under basal conditions only 2% of NKCC2 is expressed at the tubular membrane of TAL cell but in cases of intracellular chloride depletion

NKCC2 is activated and expressed at the surface of cell membrane. The activation of NKCC2 is accomplished via phosphorylation of certain threonines residues at 96, 101 and 111 positions of its amino terminus. Intracellular chloride depletion activates WNK3 which acts as an intracellular chloride sensor, in advance activated WNK3 activates SPAK/OSR1 kinases which phosphorylates NKCC2 molecule at threonines 96, 101 and 111 and promotes its trafficking to cell membrane. In cases of intracellular chloride accumulation, the above mentioned metabolic pathways cease and result in diminished NKCC2 expression at the apical cell membrane.[49,51,52]

In cases of CaSR activating mutations, the above mentioned metabolic pathways lead to diminished reabsorption of sodium chloride as well as to urinary loss of calcium and magnesium by inhibiting the paracellular reabsorption of these anions. Inactivation of the tubular lumen ion transporters leads to the phenotype of Bartter's syndrome. Functional suppression of parathyroid gland cells also leads to decreased levels of serum PTH. All these defects lead to the mixed phenotype of ADH and Bartter's syndrome type V.

CaSR is activated not only by the divalent and trivalent cations but also by positively charged organic molecules such as polyamines, aminoglycoside antibiotics, protamine and polyarginine. Aminoglycoside antibiotics especially gentamycin, amikacin and neomycin have been described as causes of Bartter's syndrome type V.[46,53]

Gitelman's syndrome

Gitelman's syndrome also known as familial hypokalemia-hypomagnesaemia is one of the most frequent hereditary tubulopathies and is characterized by hypokalemic metabolic alkalosis with significant hypomagnesemia and hypocalciuria. The disease is caused by mutations of the *SLC12A3* gene located in chromosome 16q13 (OMIM 263800) and is transmitted as an autosomal recessive trait. The *SLC12A3* gene encodes the NCC protein which is expressed in the initial part of the DCT and its function is suppressed by thiazide diuretics.[5,12]

There are more than 180 mutations affecting the whole protein gene, which include missense, nonsense, frame shift and splice site ones. The most frequent abnormality found in mutated protein is lack of glycosylation which inhibits protein trafficking to cell membrane and remains inactive in endoplasmic reticulum or does not exhibit normal NaCl transport when expressed at tubular lumen cell surface. In most patients, mutations of the *CLCNKB* gene have been identified and hence it is necessary to seek mutations affecting this gene in phenotypes with Gitelman's syndrome with no mutations affecting the *SLC12A3* gene.[5,54,55]

The clinical course of the disease is generally mild and is usually presented beyond the age of six years. Most of the patients are diagnosed in adult life. The most frequent symptoms are salt craving, muscle cramps, muscle weakness and aches, paresthesias, fatigue, dizziness, nocturia and polydipsia. Tetany may be present especially during episodes of fever or diarrhea as a result of hypomagnesaemia. The most frequent laboratory abnormalities which need prompt treatment in emergency room are hypokalaemia and hypomagnesaemia. Q-T interval prolongation is a frequent finding among these patients, but serious cardiac arrhythmias are fortunately rare. The long-term prognosis of these patients is excellent and the majority of symptoms are easily manipulated with oral administration of magnesium chloride and potassium chloride if it is needed. Low blood pressure is easily treated by increased consumption of salt containing foods.[5,56]

The cation imbalance encountered in Gitelman's syndrome, especially the handling of calcium and magnesium by the kidney, is poorly understood to the present. Hypocalciuria and hypomagnesemia which are the hallmark of differential diagnosis between Bartter's and Gitelman's syndromes appear to exhibit a complex etiology and encompass passive and active ions movement across the nephron epithelia.

The phenotype in Gitelman's syndrome is characterized by mild renal sodium wasting and low blood pressure with marginally increased levels of aldosterone which may partially explain compensatory increased reabsorption of sodium from the distal nephron in exchange with potassium and hydrogen ions. These mechanisms may explain hypokalemia and metabolic alkalosis encountered in Gitelman's syndrome but do not explain sufficiently the renal compensation for sodium balance as well as the handling of divalent cations such as calcium and magnesium.[56]

Calcium reabsorption in the DCT is inversely related to sodium reabsorption in this segment of nephron and it is encountered also after administration of thiazide diuretics, which are used effectively in the treatment of hypercalciuric patients with nephrolithiasis. Early in 1974, Costanzo et al[57] found experimentally this unusual hypocalciuric effect of thiazide diuretics.

Before the elucidation of the underlying molecular mechanisms for reabsorption of transepithelial calcium and magnesium, the explanation for the hypocalciuric effect of thiazide diuretics and that encountered in Gitelman's syndrome was dependent on two assumptions. First, the extarcellular volume contraction caused by thiazide diuretics and salt loosing in Gitelman's syndrome lead to increased reabsorption of sodium and calcium by the proximal tubule. Second, the inhibition of Na^+ and Cl^- transport in the DCT as a consequence of NCC inactivation

decreases the intracellular chloride concentration which is responsible for membrane hyperpolarization which in turn leads to increased reabsorption of calcium via voltage gated apical membrane calcium channels. Increased intracellular calcium concentration leads to increased calcium efflux from the basolateral membrane to the interstitium via PMCA1b and CNX1 which is also activated by membrane hyperpolarization and reduced concentration of intracellular sodium.[58] The above mentioned mechanisms have never been proved experimentally in patients with Gitelman's syndrome or in experimental animals with NCC inactivation.

In 1999, Hoenderop et al[59] and Peng et al[60] cloned and characterized the molecular structure of the epithelial calcium channels which are responsible for calcium reabsorption from the small intestine and DCT epithelial cells. Two members of the TRPs family of channels namely TRPV5 and TRPV6 are responsible for calcium reabsorption in the kidney and small intestine, respectively. TRPV5 is mainly expressed in DCT and CNT epithelial cells which co-localize with the calcium transport protein calbidin-D_{28k}, CNX1 and PMCA1b. TRPV6 is expressed mainly in the epithelial cells of the proximal duodenum and co-localizes with the calcium transport protein calbidin-D9k and PMCA1b.[27]

In 2002 and 2003, two studies[61,62] investigated patients with autosomal-recessive hypomagnesemia with secondary hypocalcemia and found that certain members of the TRPs family of channels, namely TRPM6 and TRPM7, are responsible for reabsorption of transcellular magnesium in the colon and DCT epithelia. The predominant channel is TRPM6, which is expressed in cell surface and forms homotetramers or heterotetramers with TRPM7 (Fig. 3).

Reabsorption of filtered magnesium along with the nephron differs significantly from reabsorption of calcium. About 60% of filtered calcium is reabsorbed in the proximal convoluted tubule (PCT), whereas only 20% of filtered magnesium is reabsorbed in this nephron segment, conversely about 20% of calcium and 65%-70% of filtered magnesium are reabsorbed in the TAL. In the PCT and TAL, reabsorption of calcium and magnesium is accomplished via a passive paracellular way. About 10% of each anion is reabsorbed via an active transcellular way in the DCT, and because no magnesium is reabsorbed beyond this nephron segment, the fine tuning of magnesium homeostasis is accomplished in the DCT.[27,58]

Experiment on a mouse model lacking the expression of the *NCC* gene ($NCC^{-/-}$ mice) revealed marked structural changes in the DCT. Loffing et al[63] found that this mouse model exhibits the phenotype of Gitelman's syndrome with hypokalemic metabolic alkalosis, elevated aldosterone levels, hypomagnesemia and hypocalciuria. They found pronounced atrophy in the early DCT segment, late normal DCT segment and pronounced CNT hypertrophy. Western blot analysis and immunohistochemistry revealed normal expression of NKCC2 in the TAL, absence of NCC expression in the early DCT, weak expression of ENaC and high expression of TRPV5 and calbidin-D_{28k} in the late DCT. In contrast, they found increased expression of all ENaC subunits in tubular membrane of CNT, suggesting increased sodium transport in this nephron segment.

Moreover, using clearance and micropuncture techniques, they found that compared with wild type animals, NCC deficient mice exhibited reduced glomerular filtration rate and increased passive reabsorption of sodium and calcium in the proximal tubule but they found no difference in reabsorption of calcium in the DCT. They concluded that the adaptation use of the nephron to reduced NaCl reabsorption in the DCT is accomplished by increased passive reabsorption of sodium in proximal tubule and enhanced active reabsorption of sodium in CNT under the influence of increased levels of aldosterone. Hypocalciuria attributed to increased fractional reabsorption of calcium in the proximal tubule.[63]

In another study, Nijenhuis et al[64] investigated calcium and magnesium homeostasis in transgenic mice with *TRPV5* gene ablation ($Trpv5^{-/-}$) as well as *NCC* gene ablation ($NCC^{-/-}$) 6 days after acute and chronic administration of thiazide diuretics. They found that $Trpv5^{-/-}$ mice exhibited about six-fold higher urine calcium excretion than wild type animals, but chronic administration of thiazide diuretics produced a significant reduction in urine calcium excretion among in wild type and $Trpv5^{-/-}$ mice. This finding suggests that hypocalciuria observed after administration of thiazide diuretics is not related to reduction in active reabsorption of calcium in the DCT.

By using real-time quantitative PCR analysis and immunohistochemistry, researchers[64] found that chronic administration of thiazide diuretics did not alter the expression of TRPV5 in wild type and KO animals, but increased the expression of sodium hydrogen exchanger 3 in the proximal tubule and decreased the expression of TRPM6 in the DCT in both animal models. Decreased expression of TRPM6 was also observed in $NCC^{-/-}$ mice.

Micropuncture studies revealed that fractional reabsorption of sodium, fluid and calcium in the proximal tubule was increased in the same animals treated with thiazide diuretics. Fractional calcium delivery in the distal nephron was significantly decreased in thiazide diuretics-treated animals compared with controls (4% *vs*. 10% respectively).[64]

Conclusions

The chronic administration of thiazide diuretics and NCC inactivation in Gitelman's syndrome or defective NCC function produce salt loosing and extarcellular volume contraction which results in increased aldosterone levels. The adaptive mechanisms by which the kidney compensates for these alterations include increased proximal tubular reabsorption of sodium and calcium and decreased reabsorption of magnesium by the distal nephron. Increased aldosterone levels are responsible for increased excretion of potassium in exchange for sodium in the distal DCT and CNT. Moreover, significant structural changes of the distal nephron such as early DCT atrophy and CNT hypertrophy in Gitelman's syndrome are superimposed. The mechanisms by which TRPM6 is down-regulated remain obscure.

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