

# The importance of the thick ascending limb of Henle's loop in renal physiology and pathophysiology

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**Abstract:** The thick ascending limb (TAL) of Henle's loop is a crucial segment for many tasks of the nephron. Indeed, the TAL is not only a mainstay for reabsorption of sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), and divalent cations such as calcium ( $\text{Ca}^{2+}$ ) and magnesium ( $\text{Mg}^{2+}$ ) from the luminal fluid, but also has an important role in urine concentration, overall acid-base homeostasis, and ammonia cycle. Transcellular  $\text{Na}^+$  transport along the TAL is a prerequisite for  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  homeostasis, and water reabsorption, the latter through its contribution in the generation of the cortico-medullary osmotic gradient. The role of this nephron site in acid-base balance, via bicarbonate reabsorption and acid secretion, is sometimes misunderstood by clinicians. This review describes in detail these functions, reporting in addition to the well-known molecular mechanisms, some novel findings from the current literature; moreover, the pathophysiology and the clinical relevance of primary or acquired conditions caused by TAL dysfunction are discussed. Knowing the physiology of the TAL is fundamental for clinicians, for a better understanding and management of rare and common conditions, such as tubulopathies, hypertension, and loop diuretics abuse.

**Keywords:** TAL, sodium handling, potassium handling, acid-base homeostasis, urine concentration

## Physiology of the thick ascending limb (TAL) Morphological features of TAL cells

The loop of Henle is a highly specialized nephron site, with two peculiar properties, its extreme heterogeneity and its anatomic configuration. It is composed of the pars recta of the proximal tubule (PT; thick descending limb), the thin descending and ascending limbs, the TAL, and the macula densa.<sup>1</sup> The descending limb penetrates the medulla, where an increasing interstitial osmotic gradient is guaranteed by the noticeable addition of  $\text{NaCl}$  through the water impermeable TAL, amplified by the hairpin-shaped loop of Henle's, with the so-called countercurrent multiplier system.<sup>2,3</sup> A similar arrangement of the surrounding vessels prevents osmotic gradient dissipation.<sup>4</sup> Electron microscopy studies demonstrated the presence of two distinct cell types in the TAL, differing in the configuration of the apical membrane: rough-surface cells (R cells), characterized by abundant apical microvilli, and smooth-surface cells (S cells) with no microprojections, but with several subapical vesicles.<sup>5</sup> The former is abundant in the cortex, and the latter in the medulla. Functional studies have demonstrated that R cells have high apical and low basolateral  $\text{K}^+$  conductance, while S cells have low apical and high basolateral  $\text{K}^+$  conductance, in hamster.<sup>6</sup> The main differences in salt absorption between the two cell types are unclear. Both cell types show similar mechanisms of

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transport; however, Nielsen et al demonstrated that S cells showed an intense labeling of the Na-K-2Cl cotransporter (NKCC2) on subapical vesicles, that may serve as a reservoir that can be recruited based on homeostatic needs.<sup>7</sup>

## Transcellular and paracellular salt absorption along the TAL

The loop of Henle is responsible for the reabsorption of ~40% of filtered Na<sup>+</sup>, mostly in the TAL.<sup>3</sup> Here, Na<sup>+</sup> entry from the lumen into the cells occurs mainly via the luminal electroneutral Na<sup>+</sup>,K<sup>+</sup>,2Cl<sup>-</sup> cotransporter, NKCC2, encoded by the *SLC12A1* gene.<sup>8</sup> This cotransporter mediates secondary active Na<sup>+</sup>, K<sup>+</sup>, and 2Cl<sup>-</sup> uptake across the apical membrane.<sup>9</sup>

The protein belongs to the family of solute carrier family 12, that includes two isoforms in humans encoded by two genes, *SLC12A1* (NKCC2) and *SLC12A2* (NKCC1).<sup>3</sup> While NKCC1 is widely expressed in several organs and tissues, NKCC2 is exclusively localized along the TAL.<sup>10</sup> Once reabsorbed from the lumen, Na<sup>+</sup> exits the cell via the sodium pump; ClC-Ka and ClC-Kb channels, with their Barttin subunit, mediate Cl<sup>-</sup> exit from the basolateral side, participating in the electronegative blood side.<sup>11</sup> The importance of ClC channels in human physiopathology is highlighted by the evidence that mutations in every channel lead to human disease.<sup>12</sup> Finally, K<sup>+</sup> ions are delivered back to the lumen through the apical renal outer medulla K<sup>+</sup> channels (ROMK). These channels perform a dual crucial role in the TAL: first, they ensure K<sup>+</sup> recycling to the lumen, essential for salt reabsorption; second, they set a positive transepithelial voltage, that drives paracellular reabsorption of cations.<sup>13,14</sup>

Additional Na<sup>+</sup> reabsorption is driven by the generated electrical field through the paracellular way; moreover, other cations (Ca<sup>2+</sup> and Mg<sup>2+</sup>) are reabsorbed by this route.

There is no doubt that the bulk of Ca<sup>2+</sup> reabsorption along the TAL occurs through the paracellular pathway, following the electrochemical gradient. NKCC2 and in particular ROMK generate the “driving force” for paracellular cation transport. Interestingly, the evidence that paracellular transport is regulated by the calcium sensing receptor (CaSR) signaling in the absence of changes in transepithelial voltage suggested that paracellular permeability may vary, with consequent changes in divalent cations reabsorption.<sup>15</sup> The CaSR, expressed on the basolateral membrane of TAL cells, has been shown to modulate Ca<sup>2+</sup> absorption through two mechanisms: 1) by inhibiting ROMK activity, thus leading to a decreased lumen-positive voltage;<sup>16</sup> and 2) by modulating tight junction (TJ) structure.<sup>17,18</sup> The latter is the main determinant of paracellular permeability. Claudins are some

of the main components of TJ. Along the TAL, several claudins are expressed, including claudin-16, 19, 10, 3, and 18.<sup>19</sup> Their role in divalent cations absorption has been recently highlighted by the discovery of salt losing phenotype associated with their dysfunction due to genetic mutations.<sup>20</sup> However, several evidences suggest that a significant component of Ca<sup>2+</sup> reabsorption occurs also via transcellular pathway.<sup>21</sup> The presence on the basolateral membrane of the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX) and the Ca<sup>2+</sup>ATPase (PMCA) confirms the presence of a basolateral exit, and suggests the possible presence of a luminal route for Ca<sup>2+</sup> entry.<sup>22</sup>

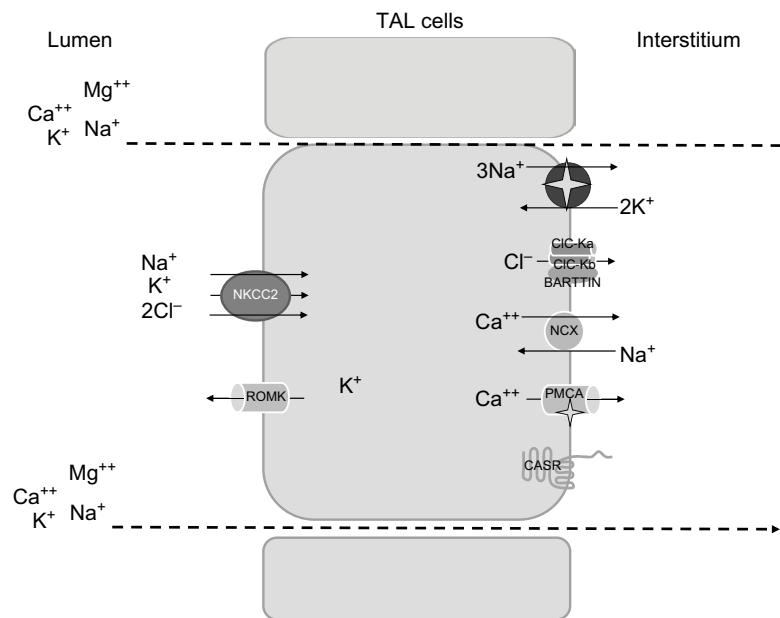
Micropuncture experiments have demonstrated that nearly 60% of the filtered Mg<sup>2+</sup> is reabsorbed in the TAL.<sup>23</sup> Passive paracellular transit is the main route, and it is driven by the lumen-positive voltage. The importance of claudin-16 (paracellin 1) and claudin-19 in Mg<sup>2+</sup> homeostasis has been pointed out in the recent years, given the association of their mutation with familial hypomagnesemia.<sup>24</sup>

The synergic activity of the main transporters and channels involved in salt absorption (NKCC2, ROMK, the chloride channel Kb [also known as ClC-Kb], with the Barttin subunit) and the integrity of TJs are the prerequisite to prevent electrolytes imbalance (Figure 1, Table 1). The loss of function of any of those proteins leads to a salt losing phenotype. As salt absorption in the TAL is under the control of hormones, local mediators, and several intracellular signaling pathways, it is not uncommon that impaired salt absorption is secondary to these dysfunctions. As an example, activating mutations of *CASR* have been described to lead to salt losing nephropathy;<sup>25</sup> similarly, dysfunction of uromodulin, Ste20-related proline-alanine-rich kinase (SPAK), and oxidative stress response kinase (OSR1) have been shown to influence NKCC2 activity and to determine fluid and electrolytes imbalance.<sup>26-28</sup>

## Generation of the cortico-medullar osmotic gradient

One of the major functions of TAL is the creation of the cortico-medullar osmotic gradient. In humans, interstitial osmolality progressively increases from the cortex, where it is ~290 mOsm/kg, to the tip of the medulla, where it reaches 1200 mOsm/kg.<sup>2</sup> The U-shaped arrangement of the two branches of the loop of Henle, the different permeabilities of the descending and ascending limbs to Na<sup>+</sup> and water, and the active Na<sup>+</sup> reabsorption in TAL are fundamental factors in the creation of the medullary osmotic gradient.<sup>4</sup>

Fluid entering the descending limb from the PT is isotonic with the plasma and the surrounding interstitium. The renal



**Figure 1** Schematic representation of TAL cells, illustrating the major transcellular proteins involved in salt reabsorption.

**Abbreviations:** TAL, thick ascending limb of the loop of Henle; ROMK, renal outer medulla K<sup>+</sup> channels; CaSR, calcium sensing receptor; NCX, Na<sup>+</sup>/Ca<sup>2+</sup> exchanger; PMCA, plasma membrane Ca<sup>2+</sup> ATPase; NKCC2, Na-K-2Cl cotransporter.

**Table 1** Main channels, transporters, and other proteins relevant in salt absorption along the TAL

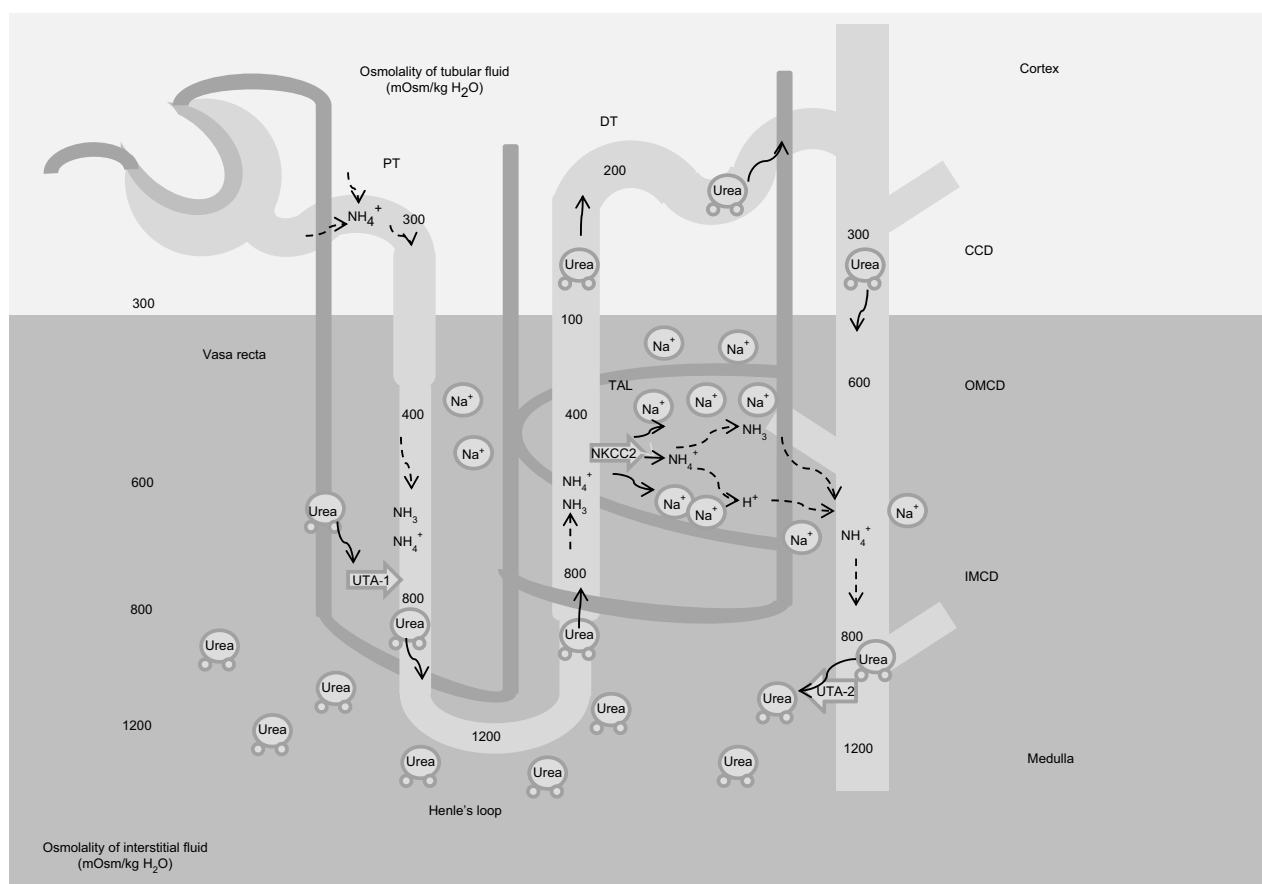
Genetic locus	Protein name	Cellular localization	Main biological functions	Correlated genetic human disease
SLC12A1	NKCC2	Apical site	Na <sup>+</sup> , Cl <sup>-</sup> , K <sup>+</sup> transport; ammonia transport	BS type I
KCNJ1	ROMK	Apical site	K <sup>+</sup> recycling to the lumen	BS type II
CLCNKB	CIC-Kb	Basolateral site	Cl <sup>-</sup> exit from the cells to the interstitium	BS type III
BSND	Barttin	Basolateral site	Cl <sup>-</sup> exit from the cells to the interstitium	BS type IV
CASR	CaSR	Basolateral site	Regulation of transcellular and paracellular salt absorption	BS type V
UMOD	Uromodulin (Tamm-Horsfall protein)	Apical site	Regulation of NKCC2 phosphorylation; regulation of ROMK surface expression	UAKD

**Abbreviations:** UAKD, uromodulin-associated kidney disease; TAL, thick ascending limb of the loop of Henle; ROMK, renal outer medulla K<sup>+</sup> channels; CaSR, calcium sensing receptor; NKCC2, Na-K-2Cl cotransporter; BS, Bartter syndrome.

epithelium along the descending limb is highly permeable to water but not to solutes.<sup>29</sup> Here, the increasing interstitial osmolality from the cortex to the medulla provides the driving force for water reabsorption (Figure 2).<sup>30–32</sup> Thus, water diffuses from the lumen to the interstitium along the descending limb and luminal osmolality rises progressively up to the tip of Henle's loop. Active Na<sup>+</sup> and Cl<sup>-</sup> reabsorption along the TAL serves two roles: 1) first, it dilutes the luminal fluid, as it is impermeable to water, so the solutes that accumulate into the interstitium are not followed by the osmotic flow of water; 2) second, by adding Na<sup>+</sup> and Cl<sup>-</sup> to the interstitium, it provides a strong contribution in the generation of the interstitial osmotic gradient in the outer medulla.<sup>33</sup>

While Na<sup>+</sup> is fundamental in the determination of the osmotic gradient in the outer medulla, in the inner medulla

urea provides a strong contribution, thanks to its reabsorption from the lumen along the collecting duct (CD), and the recycling along the descending limb.<sup>2</sup> Similarly to the two branches of Henle's loop, the descending and ascending vasa recta are arranged in a specific anatomic relationship. The countercurrent flow configuration establishes an axial osmolality gradient that is distributed along the cortico-medullary axis.<sup>34</sup> Osmotic equilibration is achieved through a combination of water absorption and solute secretion, as vasa recta are permeable to water, urea, and sodium. However, the descending vasa recta, penetrating in the medulla, lose water and gain solutes, while the ascending vasa recta show the opposite behavior, preventing dissipation of the osmotic gradient.<sup>35</sup> Conditions that decrease medullary flow, such as dehydration, favor urine concentration as blood transit into



**Figure 2** Mechanism of urine concentration.

**Note:** Contribution of  $\text{Na}^+$ , urea, and  $\text{NH}_4^+/\text{NH}_3$  in the generation of the interstitial medullary hyperosmolality.

**Abbreviations:** PT, proximal tubule; DT, distal tubule; UTA2, urea transporter; NKCC2, Na-K-2Cl cotransporter; TAL, thick ascending limb of the loop of Henle; CCD, cortical collecting duct; OMCD, outer medullary collecting duct; IMCD, inner medulla collecting duct.

the vasa recta has more time to achieve osmotic equilibration with the hypertonic interstitium. Conversely, an increased medullary flow decreases the urine concentrating ability.<sup>34,36</sup>

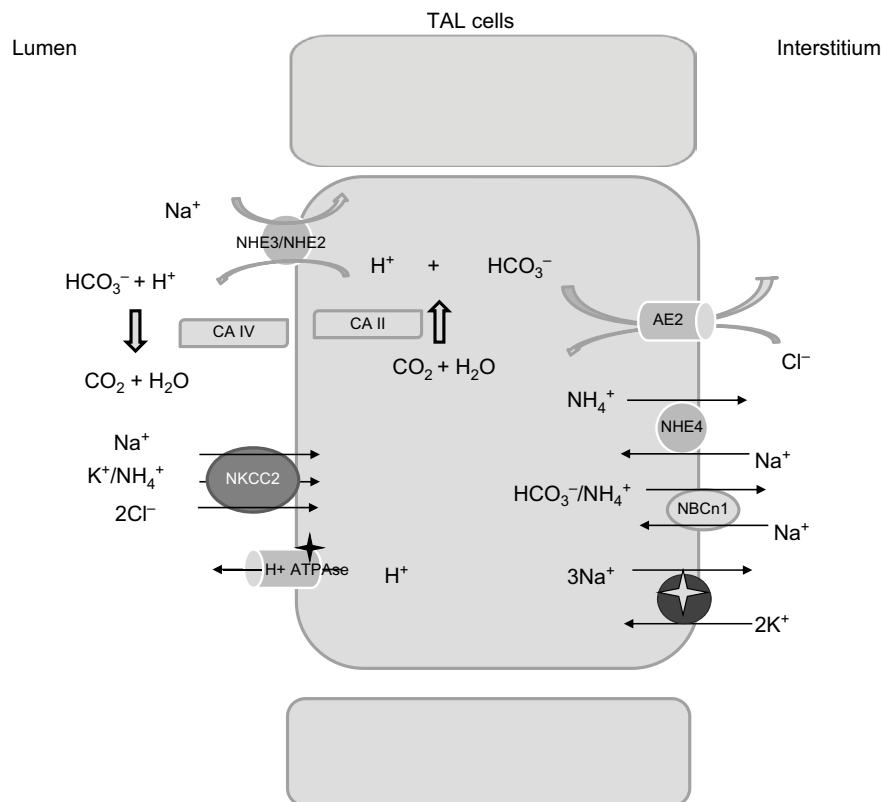
## Acid–base homeostasis

The role of PT in bicarbonate reabsorption is well established.<sup>37–39</sup> However, other downstream segments contribute to bicarbonate reabsorption. The loop of Henle reabsorbs a significant fraction (nearly 15%) of the filtered bicarbonate.<sup>40</sup> In vivo studies have demonstrated that the descending limb of Henle's loop has low bicarbonate permeability in rats. In contrast, the final segment of the PT (S3 portion) has been shown to be able to reabsorb bicarbonate. Under physiologic conditions, its contribution in bicarbonate reabsorption is modest, as it is actively reabsorbed along the early PT segments and its luminal concentration is low.

Micropuncture studies have shown that bicarbonate concentration increases significantly at the tip of Henle's loop, most probably as an indirect effect resulting from luminal fluid concentration due to water reabsorption along the descending

limb.<sup>41</sup> In the TAL, bicarbonate is reabsorbed via the transcellular pathway; the main mechanism resembles bicarbonate reabsorption in the PT through  $\text{Na}^+/\text{H}^+$  exchanger (NHE) activity.<sup>42</sup> Both NHE2 and NHE3 isoforms have been localized to the luminal membrane.<sup>43</sup> The contribution of NHE2 activity is considered low, as the addition of NHE2 knockdown in mice did not result in an overt acid–base disturbance compared with NHE3 knockout (KO) mice.<sup>44</sup> Perfusion studies, in vivo and ex vivo, showed that bicarbonate reabsorption requires carbonic anhydrase, and is stimulated by bumetanide.<sup>41</sup> Interestingly, de Bruijn et al have demonstrated that furosemide promotes luminal fluid acidification in the TAL by lowering the intracellular  $\text{Na}^+$  concentration, which in turn increases the driving force for NHE3 activity.<sup>45</sup> Bicarbonate exit from the cells is mediated by the  $\text{Cl}^-/\text{HCO}_3^-$  exchanger 2 (AE2) (Figure 3).<sup>46</sup> Interestingly, at the luminal site, the presence of the  $\text{Cl}^-/\text{HCO}_3^-$  exchanger 1 (AE1) has been shown in rats, that has been proposed to participate in  $\text{Na}^+$  reabsorption by coupling the  $\text{Na}^+/\text{H}^+$  activity.<sup>47</sup>

Besides NHE3, additional molecules contribute to bicarbonate reabsorption in the TAL. Functional studies have



**Figure 3** Relevant proteins involved in acid–base homeostasis in the TAL.

**Abbreviations:** NHE2/NHE3, isoforms 2 and 3 of the  $\text{Na}^+$ ,  $\text{H}^+$  exchangers; CA IV, isoform IV of carbonic anhydrase; CA II, isoform II of carbonic anhydrase; NKCC2, Na–K–2Cl cotransporter; AE2,  $\text{Cl}^-/\text{HCO}_3^-$  antiporter; TAL, thick ascending limb of the loop of Henle.

suggested the presence of an active  $\text{H}^+$ -ATPase, and immunohistochemical analysis has reported the presence of proton ATPase along the TAL.<sup>48</sup> Its role in bicarbonate reabsorption is not considered as consistent as a  $\text{Na}^+/\text{H}^+$  exchanger under physiological conditions.

Changes in acid–base balance modulate the rate of bicarbonate reabsorption along the TAL: both acute and chronic metabolic acidosis increase while metabolic alkalosis depresses bicarbonate reabsorption.<sup>49</sup> Functional studies have demonstrated that both  $\text{H}^+$ -ATPase and  $\text{Na}^+/\text{H}^+$  exchanger are able to adapt to changes in the acid–base status, probably via hormonal stimulation by glucocorticoids and aldosterone.<sup>49</sup>

Ammonia handling is not of minor importance. Urine ammonia excretion derives mainly from renal ammoniogenesis, rather than glomerular filtration.<sup>50</sup> It is produced from glutamine in the PT as ammonium ion ( $\text{NH}_4^+$ ) and is released to the luminal fluid. TAL has a crucial role in ammonia reabsorption; this process occurs via NKCC2, at the  $\text{K}^+$  binding site.<sup>51</sup>  $\text{K}^+/\text{NH}_4^+$  exchange and conductive  $\text{K}^+$  transport have also been described in the TAL, but their contribution is less significant compared with NKCC2.<sup>36</sup> Basolateral exit is mediated by the  $\text{Na}^+/\text{NH}_4^+$  exchanged via NHE4, the  $\text{Na}^+$ -

bicarbonate NBCn1 cotransporter, and by a  $\text{Cl}^-$ -dependent pathway.<sup>51–53</sup> Interstitial ammonia is at least in part passively recycled into the thin descending limb of Henle's loop, predominantly as ammonia ( $\text{NH}_3$ ). This recycling increases the axial ammonia gradient, paralleling the increasing tonicity of the interstitial fluid.<sup>54</sup> Ammonia is then secreted into the luminal fluid along the CD, by the ammonia-specific transporters, Rhesus glycoproteins Rhbg and Rhcg.<sup>55</sup>

## Pathophysiology of TAL dysfunction

Inherited and acquired conditions leading to an imbalance in salt reabsorption along the TAL have a significant impact on human pathophysiology.

Several water and electrolytes disturbances are attributed to TAL dysfunction. TAL hypofunction is responsible for water and electrolytes wasting, while increased salt absorption is considered a contributing factor in the pathogenesis of hypertension.

## Bartter syndrome (BS)

BS is a rare disorder caused by genetic mutations leading to impaired salt reabsorption along the TAL. Five genetic

BS subtypes have been described; BS type I is caused by mutations in *SLCA12A1* (NKCC2) gene, while BS type II is associated to *KCNJ1* (ROMK) mutations; type III and IV are caused by *CLCNKB* (CIC-Kb) and *BSND* (Barttin subunit) genetic aberrations; and BS type V results from activating mutation of the *CASR* (CaSR).<sup>56</sup> Animal models of BS have provided a significant contribution in understanding the mechanism of the disease. Both NKCC2 and ROMK null mice are characterized by early lethality.<sup>57,58</sup> The recessive mutant mouse line *Slc12a1(I299F)* resembled the features of patients suffering from type I BS, including polyuria, metabolic alkalosis, and hypercalciuria.<sup>59</sup> Interestingly, ROMK null mice develop transient hyperkalemia after birth, consistent with ROMK hypofunction in the CD, where the channel mediates  $K^+$  secretion.<sup>60</sup> Later, infants develop classic features of BS, including hypokalemia, sustained by  $K^+$  secretion along the CD mediated by the flow-dependent maxi- $K^+$  channels, despite ROMK deficiency. All BS patients share similar clinical features and biochemical abnormalities, including fluid loss leading to low-normal blood pressure, hypokalemia, and metabolic alkalosis. Often, hypercalciuria occurs, while  $Mg^{2+}$  loss is generally balanced by increased reabsorption through downstream nephron sites, mainly the distal tubule (DT).<sup>61,62</sup> Fluid wasting causes the activation of the renin–angiotensin–aldosterone system (RAAS) axis, while  $Ca^{2+}$  loss may lead to secondary hyperparathyroidism.<sup>63</sup> Two major clinical forms of BS have been described, the antenatal (also known as hyperprostaglandin E syndrome) and the classic variant. The antenatal form is characterized by polyhydramnios and growth retardation in utero, with severe hypokalemia and fluid loss requiring intensive treatment at birth. This form is generally characteristic of type I, II, and IV. The latter is the only one that is associated with hearing loss, due to the presence of the Barttin subunit in the inner ear. The classic form tends to be less severe, with a late onset, generally in childhood.<sup>63</sup> As a result of chronic volume depletion, increased PT urate reabsorption and consequent hyperuricemia may occur.<sup>64,65</sup> Type III BS has a variable phenotype. It is linked to the *CLCNKB* gene, expressed on the basolateral site of both TAL and DT. The disease may have features of BS (either antenatal or classic) and/or Gitelman syndrome.<sup>66</sup> CIC-K2 deficient mice exhibit salt losing phenotype, with compensatory increased RAAS axis, hypotension, and increased prostaglandin E2 generation; interestingly, the mice show a blunted response to both furosemide and thiazides, indicating a combined defective salt absorption along both TAL and DT.<sup>67</sup> Activating mutations of the CaSR have been associated with hypocalcemia

and hypercalciuria, and sometime hypomagnesemia.<sup>68</sup> The pathogenesis of  $Ca^{2+}$  and  $Mg^{2+}$  wasting is multifactorial in this condition. CaSR is a well-known modulator of parathyroid hormone (PTH). In the presence of high plasma  $Ca^{2+}$  levels, it inhibits PTH secretion. Gain of function mutation in the *CASR* has been shown to determine autosomal dominant hypoparathyroidism, with consequent effects on serum levels of  $Ca^{2+}$ , phosphate, and  $Mg^{2+}$ .<sup>69</sup> However, the activation of CaSR in the kidney is believed to contribute to  $Ca^{2+}$  wasting, as suggested by the evidence that patients carrying *CASR* mutations have more severe urine  $Ca^{2+}$  loss compared with patients with primary hypoparathyroidism.<sup>70</sup>

Recently, cases of some male infants showing features of transient antenatal BS, associated with mutations in the *MAGE-D2* gene, have been reported.<sup>71</sup> The gene product is expressed in the DT and in the TAL in both adult and fetal human kidney. Immunostaining studies revealed reduced NKCC2 and  $Na^+-Cl^-$  cotransporter (NCC) apical abundance in one of these patients, with increased NKCC2 cytoplasmic retention. In vitro, MAGE-D2 promotes trafficking to the apical membrane of both NCC and NKCC2.

## Familial hypomagnesemia

Claudins are transmembrane proteins involved in intercellular adhesion and paracellular barrier formation.<sup>20</sup> In mammals, over 24 claudin genes have been described; some genes exhibit peculiar tissue-specificity expression, others are expressed in several organs and tissues. In the kidney, several claudins have been mapped along the nephron.<sup>72</sup> In 1999, Simon et al discovered a mutation in a gene encoding a protein belonging to this family, *CLDN16* (encoding claudin-16, also named paracellin), causing familial hypercalciuric hypomagnesemia (FHHNC).<sup>73</sup> Recessive mutations of *CLDN16* and *CLDN19* (encoding claudin-19) are the most common causes of FHHNC.<sup>20</sup> The disease is believed to be the result of a deficient reabsorption of divalent cations in the TAL due to a defective paracellular barrier. This defect results in increased fractional excretion of  $Ca^{2+}$  and  $Mg^{2+}$ , leading to hypocalcemia and secondary hyperparathyroidism and sometimes to hypomagnesemia.<sup>74</sup> Nephrocalcinosis is the leading cause of renal failure. Patients with *CLDN19* mutations will also exhibit severe ocular involvement.<sup>75</sup> Interestingly, the role of claudin-10 in salt absorption along the TAL has been recently highlighted. Breiderhoff et al showed that claudin-10 deficiency affected paracellular permeability to monovalent and divalent cations in the TAL, causing nephrocalcinosis and hypermagnesemia.<sup>76</sup> Its role in TAL salt absorption has been confirmed also in humans by the study of Bongers et al.<sup>77</sup>



They described two patients carrying *CLDN10* mutations with a hypokalemic salt losing nephropathy, with hypermagnesemia. Accordingly, it has been recently shown that patients suffering from the so-called HELIX syndrome (characterized by hypohidrosis, salt losing nephropathy, lacrimal gland dysfunction, ichthyosis, and xerostomia) carried biallelic mutations of *CLDN10* gene. Functional studies demonstrated that salt wasting depended on impaired salt absorption along the TAL.<sup>78</sup> The role of claudins in salt homeostasis is further supported by a genome-wide association study demonstrating that claudin-14 variants are a risk factor for hypercalciuric nephrolithiasis.<sup>79</sup> In addition, Corre et al have recently described a genome-wide association study demonstrating that claudin-14 variants influence differential urine excretion of  $Mg^{2+}$  and  $Ca^{2+}$ , suggesting that there is still a lot to be discovered in paracellular electrolyte handling along the distal nephron.<sup>80</sup>

### CNNM2 (hypomagnesemia with seizures and mental retardation)

The *CNNM2* gene is mainly expressed in the kidney, brain, and lung in mammalian tissues and performs the function of a divalent cation transporter, including  $Mg^{2+}$ .<sup>81</sup> The protein has been localized on the basolateral membrane of both TAL and DT cells in human kidney sections. The presence along the DT is not surprising, as transcellular  $Mg^{2+}$  reabsorption from the lumen has been largely proven. The presence in the TAL may either indicate a role in the control of paracellular  $Mg^{2+}$  absorption, or a possible presence of apical  $Mg^{2+}$  uptake, as suggested by some authors.<sup>82</sup>

Mutations in this gene cause a rare disease characterized by cognitive impairment, seizure, and hypomagnesemia. The latter is believed to result from impaired luminal  $Mg^{2+}$  reabsorption. A genome-wide association study demonstrated a correlation between *CNNM2* variants and plasma  $Mg^{2+}$  levels, supporting a significant role of *CNNM2* in human  $Mg^{2+}$  homeostasis.<sup>83</sup>

### The EAST syndrome

This is a rare recessive genetic disorder associated with mutations in the *KCNJ10* gene, encoding the  $K^+$  channel Kir 4.1.<sup>84</sup> The acronym EAST indicates the most important clinical features, including epilepsy, ataxia, senso-neural deafness, and tubulopathy. The Kir 4.1 channel is in fact expressed in several organs, including the central nervous system, the inner ear, and the nephron.<sup>85</sup> In the latter, it has been shown on the basolateral membrane of the distal nephron, from the macula densa to the early CD and in cortical TAL.<sup>72</sup>

The salt losing phenotype resembles Gitelman syndrome, with hypocalciuria and hypomagnesemia, besides metabolic alkalosis and hypokalemia. Fan et al demonstrated that the vasopressin-dependent stimulation of the 80–150 pS basolateral  $K^+$  channel in *Kcnj10* (Kir 4.1) KO mice counteracts the loss of function of Kir 4.1 in the TAL, preventing defective salt absorption at this nephron site.<sup>86</sup>

### Mitochondrial diseases

Inherited mitochondrial diseases are a heterogeneous class of clinical conditions characterized by impaired function of the mitochondrial respiratory chain, due to genetic mutation of either genomic or mitochondrial DNA.<sup>87</sup> The spectrum of clinical features is highly variable and the most common morbidities are neurologic and myopathic diseases. Some studies have described renal involvement, with a wide spectrum of tubular disorders, including Fanconi and Bartter-like syndrome; in addition, glomerular defects (focal and segmental glomerulosclerosis) and cystic diseases have been reported.<sup>88</sup> The Kearns–Sayre syndrome is a rare mitochondrial disease caused by a large deletion of mitochondrial DNA.<sup>89</sup> The clinical findings are Bartter-like syndrome, with hypokalemic metabolic alkalosis and nephrocalcinosis. Some reports described Fanconi-like phenotypes. Extrarenal features include ophthalmoplegia, cerebellar, heart, and several endocrine and metabolic dysfunctions.<sup>88</sup>

### Increased salt absorption along the TAL: a potential role in salt-sensitive hypertension

Hypertension is a major public health problem, and it is considered to be of multifactorial origin. High dietary salt intake is considered one of the major environmental factors favoring its development. However, the effect of salt intake on blood pressure varies among individuals, a phenomenon called salt sensitivity.<sup>90</sup> The molecular mechanism underlying salt sensitivity is only partially understood. A significant contribution is mainly attributable to an impaired ability to excrete  $Na^+$ . Evidences demonstrate that increased  $Na^+$  reabsorption along the distal nephron plays a crucial role in the pathogenesis of hypertension.<sup>91,92</sup> Monogenic forms of hypertension include clinical conditions characterized by increased salt absorption along the DT (Gordon syndrome) and the CD. The latter may be caused by gain of function mutations of the principal apical channel mediating  $Na^+$  entry, epithelial sodium channel (Liddle syndrome), or by the activation of mineralocorticoid receptors (autosomal dominant pseudoaldosteronism type I).<sup>93</sup> Indirect data suggest

that the TAL also has a crucial role in fluid balance and the pathogenesis of hypertension.<sup>94</sup> TAL hypofunction, as in BS, clearly leads to fluid loss and low-normal blood pressure. Animal models of salt-sensitive hypertension show increased avidity in salt absorption along the TAL. Milan hypertensive strain rats displayed increased NKCC2 activity during the induction phase of hypertension.<sup>95</sup> In Dahl salt-sensitive rats, increased TAL  $\text{Cl}^-$  uptake has been demonstrated by in vivo micropuncture studies.<sup>96</sup> In accordance with animal studies, several data suggest that also in humans TAL contributes to salt sensitivity and hypertension. African-American individuals show a much higher incidence of salt-sensitive hypertension than white people. In the first group of subjects, a lower plasma renin activity (PRA) has been shown, suggesting a primary increase in renal salt reabsorption and consequent compensatory PRA suppression.<sup>97</sup> Results of Luft et al's studies, showing a higher kaliuretic response after furosemide administration compared with white people, considered TAL as the major nephron site responsible for increased salt reabsorption in black individuals.<sup>98</sup>

In the Framingham heart study population, heterozygote genetic variants of NKCC2, ROMK, and NCC correlated with low blood pressure.<sup>99</sup> In addition, a recent genome-wide association study indicated *UMOD* gene variants as risk factors for both salt-sensitive hypertension and kidney damage,<sup>27</sup> further supporting the hypothesis of TAL contribution in salt-sensitive hypertension.<sup>100,101</sup> In fact, besides other known functions, uromodulin (*UMOD*) has been proposed to modulate salt absorption in the TAL. Graham et al showed that *UMOD*<sup>-/-</sup> mice were resistant to salt-induced changes of blood pressure,<sup>102</sup> while an independent study showed that *UMOD* overexpression increased blood pressure.<sup>102</sup> In vitro studies suggest that *UMOD* increases either NKCC2 or ROMK activities.<sup>27</sup> Moreover, the evidence that cyclosporine-induced hypertension is associated with NKCC2 upregulation further supports the hypothesis that increased avidity in salt absorption along the TAL contributes to the pathogenesis of hypertension.<sup>103,104</sup>

## Acquired conditions resulting in TAL hypofunction: electrolytes disorders, loop diuretics, bilateral ureteral obstruction

### Chronic electrolytes imbalance

Hypokalemia is known to inhibit urine concentrating ability and  $\text{Na}^+$  and  $\text{Cl}^-$  absorption along the TAL.<sup>13</sup> In vivo micropuncture studies have demonstrated that chronic hypokalemia reduced  $\text{Na}^+$  and fluid absorption in the TAL.<sup>105,106</sup> In a study by Gutsche et al, impaired salt absorption along the TAL in

rats under a low  $\text{K}^+$  diet was restored by acute  $\text{K}^+$  infusion; the evidence of impaired  $\text{Na}^+$  efflux across the peritubular membrane suggested hypokalemia-dependent inhibition of the basolateral  $\text{Na}^+/\text{K}^+$ -ATPase.<sup>107</sup> The resulting increased  $\text{Na}^+$  and fluid loading to the distal  $\text{K}^+$ -secretory segments of the nephrons, that is known to enhance  $\text{K}^+$  secretion, causes further  $\text{K}^+$  urinary excretion.<sup>105</sup> Recent studies have addressed the role of the intracellular proteins SPAK and OSR1 in electrolytes homeostasis and salt absorption along the distal nephron. Both proteins have been shown to phosphorylate and activate NKCC2 and NCC in vitro.<sup>28</sup> However, their function in vivo is controversial. In fact, SPAK null mice showed a Gitelman-like phenotype, suggesting a pivotal role of the protein in NCC regulation, rather than NKCC2, while kidney-specific OSR1 null mice had a Bartter-like phenotype, confirming its role in modulating NKCC2 function.<sup>108,109</sup>

Interestingly, total double KO mice is embryonic lethal, while constitutive SPAK KO mice with inducible *OSR1* knockdown at steady state showed mild volume contraction and hypokalemia, a less severe phenotype than expected by combined NCC and NKCC2 hypofunction. Accordingly, phospho-NKCC2 abundance was unaffected despite the absence of SPAK and OSR1; conversely, phospho-NCC abundance in response to low  $\text{K}^+$  diet was reduced, suggesting a significant role of SPAK and OSR1 in NCC regulation by  $\text{K}^+$  plasma levels.<sup>28</sup>

Extracellular  $\text{Ca}^{2+}$  has a potent inhibitory effect on salt absorption. Chronic hypercalcemia leads to urinary salt loss and fluid wasting. By binding the CaSR on the basolateral membrane of the TAL, extracellular  $\text{Ca}^{2+}$  has been shown to inhibit ROMK activity via phospholipase A2, reducing the lumen-positive voltage and consequently paracellular cation reabsorption.<sup>16</sup>

As stated earlier, recent studies suggest that CaSR regulates paracellular calcium reabsorption along the TAL without affecting the transepithelial electrical gradient. CaSR activation is supposed to enhance *CLD14* expression, leading to the downregulation of claudin-16/19, with consequent hypercalciuria. The requirement of calcineurin in the pathway regulating claudin-14 expression may provide an explanation for the cyclosporine-induced hypercalciuria.<sup>110</sup>

### Loop diuretics

Diuretics are widely used in several clinical fields. Furosemide, torasemide, and bumetanide bind NKCC2 in a reversible fashion. The resulting NKCC2 inhibition leads to the reduction in  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  absorption, an effect that overrules that the cortico-medullary osmotic gradient increases



urine output and impairs paracellular cations reabsorption. This property has predictable beneficial effects in several conditions, and loop diuretics are the main therapy in fluid retentive states and hypercalcemic conditions.<sup>111</sup> After 1 hour from the oral administration of furosemide, normal subjects experienced increased urine output and increased excretional fraction of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  in a time-dependent manner, an effect that decreases after 4–6 hours. The effect is dose-dependent, and larger doses are required when the glomerular filtration rate (GFR) decreases.<sup>112</sup> Low-dose loop diuretics rarely cause important side effects; conversely, high dosage or long-term treatments, as usually is needed in chronic conditions, result in electrolytes and acid–base imbalance. Hypokalemic metabolic alkalosis is a common side effect of loop diuretics treatment. The underlying mechanism is based on increased salt delivery to the distal nephron, that enhances  $\text{K}^+$  and  $\text{H}^+$  secretion along the CD.<sup>113</sup> Extracellular volume contraction and the activation of the RAAS axis further contribute to metabolic alkalosis maintenance. Conversely, hyponatremia is uncommon in patients taking loop diuretics compared with patients under thiazides treatment, but still this complication can occur in the presence of concomitant pathologic conditions.<sup>114</sup> By increased free water clearance, loop diuretics are expected to increase rather than decrease plasma  $\text{Na}^+$  concentration. However, high dose of loop diuretics, as often can be necessary in chronic heart failure or in advanced chronic kidney disease, can cause over-diuresis, intravascular volume depletion, and increased vasopressin secretion, worsening hyponatremia.<sup>115</sup>

### Post-obstructive syndrome

Post-obstructive diuresis is a polyuric condition resulting after the relief of bilateral urinary tract obstruction. Diuresis is considered a physiological response to counteract water and salt retention during the obstruction. However, in some patients, diuresis does not cease when the excess of volume and salt is resolved, resulting in pathologic diuresis.<sup>116</sup> Several factors have been implicated in the pathophysiology of this condition. Earlier studies from the 1950s suggested that increased urine and salt excretion was the result of reduced  $\text{Na}^+$  reabsorption, with some studies placing the site of reduced reabsorption in the PT, and others in the distal nephron.<sup>117</sup>

Micropuncture studies showed that post-obstructive diuresis resulted from impaired  $\text{Na}^+$  reabsorption at nephron sites beyond the PT in rats.<sup>118</sup> McDougal and Wright showed that  $\text{Na}^+$  absorption was decreased either in PT, paralleling the reduction of the GFR, or in the TAL, leading to increased  $\text{Na}^+$

loading to the CD in rats.<sup>117</sup> Subsequent studies have shown that polyuria following bilateral obstruction relief was associated with reduced expression of the major  $\text{Na}^+$  transporters, including NHE3, NKCC2, type II Na-Pi cotransporter, and NCC.<sup>101</sup> Some authors have demonstrated the role of reduced responsivity of the CD to vasopressin, leading to a diabetes insipidus-like syndrome.<sup>119</sup> Recent studies suggest that hydrogen sulphide ( $\text{H}_2\text{S}$ ), a gasotransmitter modulating a wide range of physiologic functions, may represent a novel therapeutic approach in this condition, given the evidence that increasing  $\text{H}_2\text{S}$  levels may reduce renal fibrosis.<sup>120,121</sup> It would be of interest to address its effect on salt absorption in this setting, considering that it has been shown to modulate urinary  $\text{Na}^+$  and  $\text{K}^+$  excretion, probably by affecting the function of both NKCC2 and Na-K ATPase.<sup>122</sup>

## Conclusion

The TAL has a central role in water and electrolyte homeostasis and acid–base balance, and its dysfunction has a crucial role in human pathophysiology.

Increased awareness and understanding of its specific functions will enable physicians to better manage acquired and inherited conditions caused by TAL-defective salt absorption, and to reduce the incidence and the complications of potentially life-threatening conditions.

## Disclosure

The authors report no conflicts of interest in this work.

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