

Dent's disease: chloride–proton exchange controls proximal tubule endocytosis

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*Comment on Novarino G, Weinert S, Rickheit G et al. Endosomal chloride–proton exchange rather than chloride conductance is crucial for renal endocytosis. *Science* 2010; 328: 1398–1401.**Keywords:** CIC-5; endosomes; receptor-mediated endocytosis; renal Fanconi syndrome

Review of the field

Receptor-mediated endocytosis in the proximal tubule

Several grams of albumin and low-molecular-weight (LMW) plasma proteins are filtered daily through the glomerular basement membrane, to be reabsorbed by proximal tubule (PT) cells [9]. These LMW proteins, which are characterized by a molecular mass lower than that of albumin (~69 kDa), include hormones, vitamin-binding proteins, enzymes, immunoglobulin light chains, drugs and toxins. Most of the filtered LMW proteins are reabsorbed and metabolized by PT cells, and the human urine is virtually devoid of plasma proteins under physiological conditions. Such a massive uptake of proteins accounts for as much as 80% of the total metabolic clearance of small proteins and peptides, and plays a key role in hormone and vitamin homeostasis [10].

The uptake of LMW proteins by PT cells essentially involves receptor-mediated endocytosis, while fluid-phase capture can be considered as quantitatively negligible. During receptor-mediated endocytosis, filtered proteins are concentrated at the apical cell surface, and further reabsorbed by clathrin-mediated endocytosis. The process requires two multiligand receptors, megalin and cubilin, that are abundantly expressed at the brush border of PT cells [10]. Ligand binding and interactions between both receptors induce their internalization into coated vesicles and their subsequent delivery to endosomes and lysosomes for ligand processing and receptor degradation or recycling. The progression along the endocytic apparatus requires a sustained vesicular acidification, which triggers receptor–ligand dissociation and modulates vesicle trafficking, endosomal fusion events, and coat formation [11]. In PT cells, the endosomal acidification is driven by the electrogenic vacuolar H⁺-ATPase (V-ATPase) requiring a countercurrent system to dissipate the positive potential and to maintain electroneutrality (Figure 1). It has long been assumed that chloride channels would provide such an electrical shunt to neutralize the H⁺ gradient [12].

Summary of key findings of the article

Dent's disease is a rare disorder of the proximal tubule caused by inactivating mutations in the endosomal chloride transporter CIC-5. The disease is characterized by defective endocytosis and manifestations of proximal tubule dysfunction (renal Fanconi syndrome) [1–3]. The primary defect in proximal tubule endocytosis has been attributed to an impaired vesicular acidification caused by the loss of endosomal Cl[−] conductance mediated by CIC-5 (chloride shunt hypothesis) [4,5]. However, recent electrophysiological studies have shown that CIC-5 is a 2Cl[−]/H⁺ exchanger rather than a Cl[−] channel [6,7]. In order to test the relevance of this exchange activity for Dent's disease, Novarino, Jentsch and colleagues [8] engineered a knock-in (KI) mouse harbouring a point mutation in a critical glutamate residue which converts the exchanger into an uncoupled Cl[−] channel that should facilitate endosomal acidification. They then compared these KI mice with the conventional knock-out (KO) mice that faithfully recapitulate the phenotype of Dent's disease. As expected, acidification of the renal endosomes from WT and KI mice was normal, but severely impaired in KO mice. However, despite normal endosomal acidification, KI mice showed the same renal phenotype as KO mice and patients with Dent's disease, including low-molecular-weight proteinuria, hyperphosphaturia and hypercalciuria. Furthermore, both the KI and KO mouse showed impaired proximal tubule endocytosis and a similar trafficking defect. Thus, proximal tubule dysfunction in Dent's disease may occur despite normal acidification of renal endosomes. These findings, which exclude the chloride shunt hypothesis, suggest a role for a reduced endosomal chloride accumulation in Dent's disease and point to the importance of chloride concentration for organelle physiology.

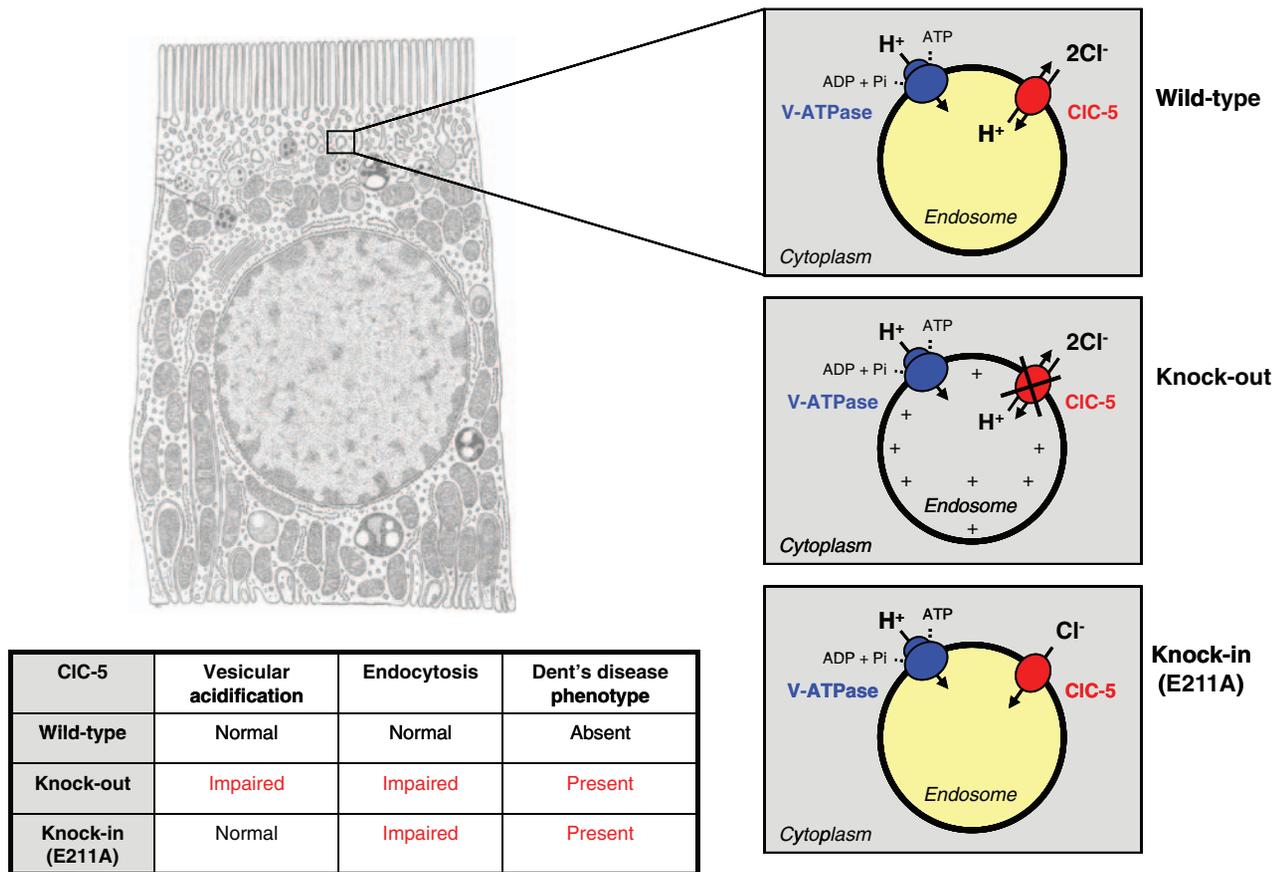


Fig. 1. The role of CIC-5 in the acidification of proximal tubule endosomes and Dent's disease. The endocytic pathway in proximal tubule cells involves coated pits and coated vesicles, followed by early endosomes that form recycling endosomes or mature to late endosomes and lysosomes. The endosomal acidification (up to pH 5.0)—that is necessary for dissociation of the ligand–receptor complex, recycling of receptors to the apical membrane, and progression of ligands into lysosomes—is achieved by ATP-driven transport of cytosolic H⁺ through the V-ATPase. The wild-type CIC-5 Cl⁻/H⁺ exchanger (upper panel) provides a counter-current for the proton pump. In the CIC-5 knock-out endosomes (middle panel), the lack of CIC-5 impairs vesicular acidification by the accumulation of luminal positive charges. In the knock-in endosomes (bottom panel), the uncoupling E211A mutation of CIC-5 results in a pure Cl⁻ channel that dissipates the potential and enables vesicular acidification. Importantly, the phenotype of Dent's disease and the defective endocytosis are similarly observed in both the knock-out and the E211A knock-in mouse models. These results indicate that Dent's disease is caused not by a defective endosomal acidification but rather by a defective endosomal accumulation of Cl⁻ ions.

The paradigm of Dent's disease

Investigations on the pathophysiology of Dent's disease and the role of CIC-5 have provided critical information on the role of chloride transporters in proximal tubule endosomes. Dent's disease (OMIM #300009) is a rare X-linked renal tubulopathy characterized by LMW proteinuria associated with hypercalciuria, which may provoke nephrolithiasis, nephrocalcinosis and renal failure [2,3]. The disease is caused by mutations in the *CLCN5* gene that encodes CIC-5, an electrogenic Cl⁻/H⁺ exchanger [1,6,7]. CIC-5 belongs to the CLC family of Cl⁻ channels/transporters that have been discovered and characterized by Jentsch and colleagues [13]. CIC-5 consists of 746 amino acids and forms diamond-shaped homodimers, each having a pore responsible for the selective coupling of Cl⁻ flux to H⁺ countertransport [14]. *In vitro* studies have demonstrated that natural mutations in CIC-5 lead to a loss of function [1]. Furthermore, genetic inactivation of the *Clcn5* gene in the mouse mimics the severe PT dysfunction observed in Dent's disease [15,16].

Pathophysiology of Dent's disease

Based on its initial characterization and its co-distribution with the V-ATPase in renal endosomes [1,15–17], CIC-5 was thought to mediate the Cl⁻ counter-current necessary for endosomal acidification. Studies in endosomes isolated from CIC-5 KO mice confirmed a decreased ATP-dependent vesicular acidification *in vitro* [4], and indeed, mice lacking CIC-5 showed a severe defect in PT endocytosis [15,16]. In addition, CIC-5 inactivation induces a generalized trafficking defect in PT cells, with loss of megalin and cubilin at the brush border and impaired lysosome biogenesis, which also contributes to defective endocytosis and urinary loss of LMW ligands and lysosomal enzymes [18].

The role played by CIC-5, however, became more difficult to understand when it was discovered that, instead of being a simple Cl⁻ channel, CIC-5 is in fact a Cl⁻/H⁺ exchanger exploiting the H⁺ gradient to move Cl⁻ ions into the endosomes [6,7] (Figure 1). To better understand the biological role of this exchange activity, and its relevance

for Dent's disease, Novarino, Jentsch and colleagues [8] generated a novel KI mouse model carrying a point mutation of CIC-5 (E211A) that they had extensively characterized *in vitro*. This mutation affects a critical intra-membrane glutamate residue that is essential for the gating of the CLC exchangers [14]: by replacing this glutamate by an alanine, CIC-5 is converted into a pure, uncoupled Cl⁻ conductor. At variance with the lack of CIC-5 in KO mouse, this very mutation should not affect the endosomal acidification. Thus, comparative analysis of the renal phenotype in the KO and KI mouse models should provide a strong indication about the role of the Cl⁻/H⁺ exchange in the pathophysiology of Dent's disease. Indeed, Novarino *et al.* [8] showed that vesicular acidification was similar in WT and KI mice, contrasting with a severe impairment in KO mice. However, despite the normal endosomal acidification, KI mice showed the same renal phenotype as KO mice and patients with Dent's disease, including LMW proteinuria, glucosuria, hyperphosphaturia and hypercalciuria. Furthermore, both the KI and KO mouse showed a similar impairment in PT endocytosis, with reduced levels of the endocytic receptors megalin and cubilin and internalization of the sodium-phosphate co-transporter NaPi-2a indicating a trafficking defect (Figure 1).

Thus, PT dysfunction in Dent's disease may occur despite normal acidification of renal endosomes, which excludes the chloride shunt hypothesis. Instead, the disease appears to be caused by defective exchange activity, i.e. uncoupling of Cl⁻ from H⁺ gradients and defective endosomal Cl⁻ accumulation (Figure 1). CIC-5 may drive endosomal acidification independently of the V-ATPase, for instance by exchanging cytosolic H⁺ for intravesicular Cl⁻ in early endosomes [7]. Novarino *et al.* suggest that the 2Cl⁻/H⁺ exchange maintains high vesicular Cl⁻ concentration not only during active acidification but also under steady state [8]. This hypothesis is supported by a companion paper by Weinert *et al.*, who demonstrate lower Cl⁻ concentration in lysosomes harbouring an uncoupling mutation in CIC-7 despite normal lysosomal pH [19]. At this stage, the role of the vesicular Cl⁻—regulation of other transport systems, interaction with other proteins involved in the organelle or importance for vesicle recycling—remains speculative.

Relevance for the practising nephrologist

The family of CLC proteins is important for nephrologists, as a pure Cl⁻ channel such CIC-Kb is involved in salt-losing tubulopathies affecting the distal nephron (Bartter and Gitelman syndromes), whereas the Cl⁻/H⁺ exchanger CIC-5 is involved in a generalized PT dysfunction (Dent's disease) [3,13].

Dent's disease illustrates how investigations of a rare disorder can give insights into fundamental biological processes that are clinically relevant. The disease stresses the importance of PT endocytosis, essential for the metabolism of small proteins and the homeostasis of hormones and vitamins. Defective PT endocytosis can readily be de-

tected by LMW proteinuria. The consequences of such an endocytic defect are not small, since cells lacking CIC-5 show dedifferentiation and oxidative stress [20], and progression to ESRD occurs in 30–80% of affected males [2]. The work of Novarino *et al.* also illustrates the importance of identifying the structure of a molecule, including critical residues involved in gating, as well as the power of genetic engineering in the mouse [8]. Finally, these data provide new insights into the question of the endosomal acidification, which has puzzled cell biologists for decades. In particular, they point to the physiological requirement of chloride along the endocytic apparatus.

Important questions remain: what is the role of intravesicular Cl⁻ concentration? And, how does this affect membrane traffic in PT cells? What is the relation between defective CIC-5/endocytosis and the hypercalciuria in Dent's disease? What is the role of other Cl⁻ channels and transporters [21] present in endosomes? Will these new data be helpful to design drugs able to restore normal endocytosis and PT function?

Take-home message

Dent's disease is a disorder of the proximal tubule caused by inactivating mutations in CIC-5 that impair the endocytic uptake of ultrafiltered proteins. New data show that CIC-5 is a chloride-proton exchanger that controls the accumulation of Cl⁻ ions into renal endosomes. Reduced endosomal Cl⁻ concentration may thus impair proximal tubule endocytosis and cause renal Fanconi syndrome.

Conflict of interest statement. None declared.

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