1. Introduction

The electrogenic Na⁺-HCO₃⁻ cotransporter NBCe1, belonging to the solute carrier 4 (SLC4) family, plays essential roles in the regulation of extracellular and intracellular pH [1,2]. Consistent with an essential role of NBCe1 in bicarbonate absorption from renal proximal tubules, homozygous mutations in NBCe1 cause proximal renal tubular acidosis (pRTA) [3-11]. These pRTA patients with NBCe1 mutations invariably present with ocular abnormalities such as band keratopathy, cataract, and glaucoma, indicating that NBCe1 also plays important roles in the maintenance of ocular homeostasis [12,13]. Some pRTA patients also have migraine, suggesting that NBCe1 may also contribute to the pH regulation in the brain [10]. In addition, mice models for NBCe1 deficiency have been developed [11,14].

In this review, we try to summarize the recent data about the pathophysiological roles of NBCe1 mutations.

2. Physiological roles of NBCe1 in kidney and pancreas

There are at least five mammalian NBCe1 variants, NBCe1A through NBCe1E as shown in Figure 1 [15,16]. NBCe1B differs from NBCe1A at the N-terminus, where the first 85 amino acids of NBCe1B replace the first 41 amino acids of NBCe1A [17]. NBCe1C differs from NBCe1B at the C-terminus, where the last 61 amino acids of NBCe1C replace the last 46 amino acids of NBCe1B [18]. NBCe1D and NBCe1E, identified from mouse reproductive tract tissues, contain a deletion of 9 amino acids in exon 6 of NBCe1A and NBCe1B, respectively [16].

Among these variants, NBCe1C is predominantly expressed in brain, but its physiological roles remain speculative [18]. NBCe1B is widely expressed in several tissues including...
pancreatic ducts, intestinal tracts, ocular tissues, and brain [2,12,13,19-22]. In the basolateral membranes of pancreatic ducts NBCe1B is thought to mediate bicarbonate uptake into cells, which may be essential for the bicarbonate secretion from pancreas [23-25]. Consistent with this view, some pRTA patients with NBCe1 mutations presented with an elevated serum amylase level [3,7]. However, none of these patients presented with a distinct form of pancreatitis. Probably, other acid/base transporters such as Na⁺/H⁺ exchanger 1 (NHE1) or H⁺-ATPase in the basolateral membranes of pancreatic duct cells could at least partially compensate for the NBCe1 inactivation [26].

**Figure 1.** Structures of NBCe1 variants. Numbers of boxes indicate numbers of amino acids in N- or C-terminus. Note that NBCe1D and NBCe1E lack 9 amino acids (9-aa) in exon 6 of NBCe1A and NBCe1B, respectively. TMD: transmembrane domain.

NBCe1A is predominantly expressed in the basolateral membranes of renal proximal tubules, where it mediates bicarbonate exit from cells [2,27]. The opposite transport directions between NBCe1A in kidney and NBCe1B in pancreas may be related to the different stoichiometric ratios. Thus, NBCe1A in *in vivo* renal proximal tubules functions with 1Na⁺ to 3HCO₃⁻ stoichiometry, whereas NBCe1B in pancreatic ducts may function with 1Na⁺ to 2HCO₃⁻ stoichiometry [23,28]. However, these differences in transport stoichiometry may not be due to the intrinsic properties of NBCe1 variants, but rather reflect the environmental factors such as incubation conditions or cell types. Indeed, NBCe1A in isolated renal proximal tubules can function with either 1Na⁺ to 2HCO₃⁻ or 1Na⁺ to 3HCO₃⁻ stoichiometry depending on the incubation conditions [29-31]. Such changes in transport stoichiometry of NBCe1A can be also induced in *Xenopus* oocytes [32]. Moreover, NBCe1B may function with 1Na⁺ to 2HCO₃⁻ stoichiometry in cultured pancreatic duct cells, but may function with 1Na⁺ to 3HCO₃⁻ stoichiometry when expressed in cultured renal proximal tubular cells [33]. Regarding the electrogeniciry of NBCe1A, recent work by Chen and Boron suggests that the predicted fourth extracellular loop corresponding to amino acids 704 to 735 may have an important role [34]. They found that replacing these residues with the
corresponding residues of electroneutral Na\(^+\)-HCO\(_3\)- cotransporter NBCn1-A creates an electroneutral NBC.

Although the basolateral membranes of renal proximal tubules are known to contain several bicarbonate transporters such as Na\(^+\)-dependent and Na\(^+\)-independent Cl-/HCO\(_3\)- exchangers [35,36], NBCe1A seems to play an essential role in bicarbonate absorption in this nephron segment. Consistent with this view, the homozygous inactivating mutations in NBCe1A cause severe pRTA with the blood bicarbonate concentration often less than 10 mM [3-11]. Functional deletion of NBCe1 in mice produces even more severe acidemia with the blood bicarbonate concentration around 5 mM [11,14]. By contrast, functional deletion of Cl\(^-\)/HCO\(_3\)- exchanger AE1, which is responsible for a majority of basolateral bicarbonate exit from α-intercalated duct cells, produces only moderate acidemia in mice with the blood bicarbonate concentration around 17 mM [37]. This may probably reflect much higher bicarbonate absorbing capacity of renal proximal tubules than that of renal distal tubules.

3. NBCe1 mutations and pRTA

Until now, 12 homozygous mutations in NBCe1 have been identified in pRTA patients associated with ocular abnormalities as shown in Figure 2 [3-11].

![NBCe1 topology and pRTA-related mutations](image)

**Figure 2.** NBCe1 topology and pRTA-related mutations. Numbers in circles correspond to Q29X, R298S, S427L, T485S, G486R, R510H, W516X, L522P, N721TfsX29, A799V, R881C, and S982NfsX4. White numbers in black circles indicate mutations associated with migraine. They include eight missense mutations R298S, S427L, T485S, G486R, R510H, L522P, A799V, and R881C, two nonsense mutations Q29X and W516X, and two frame shift mutations N721TfsX29 and S982NfsX4. Except the NBCe1A-specific mutation Q29X, which is expected to yield non-functional NBCe1A but leave both NBCe1B and NBCe1C intact [4], all the other mutations lie in the common regions of NBCe1 variants. The C-terminal mutant S982NfsX4 is expected to introduce a frameshift in exon 23 and a premature stop codon for both
NBCe1A (S982NfsX4) and NBCe1B (S1026NfsX4), yielding the mutant proteins with 51 fewer amino acids than the wild-type proteins. On the other hand, this mutation abolishes the translation of NBCe1C, the C-terminal variant skipping exon 24 [10,18].

Topological analysis using the substituted cysteine accessibility method suggests that most of these mutations are buried in the protein complex/lipid bilayer where they perform important structural roles [38]. In particular, the amino acid substitution analysis revealed that Thr485 might reside in a special position, which seems to require the OH group side chain to maintain a normal conformation of NBCe1A. Based on homology modeling to the crystallized cytoplasmic domain structure of AE1, Arg586 in the C-terminal cytoplasmic domain of NBCe1A was also predicted to reside in a solvent-inaccessible subsurface pocket and to associate with Glu91 or Glu295 via H-bonding and charge-charge interactions [39]. This unusual continuous chain of interconnected polar residues may be essential for HCO$_3^-$ transporting ability of SLC4 proteins. Parker et al. recently found that in addition to a per-molecule transport defect as previous reported [7], the NBCe1 A799V mutant has an unusual HCO$_3^-$-independent conductance that, if associated with mutant NBCe1 in muscle cells, could contribute to the occurrence of hypokalemic paralysis in the affected individual [40,41].

Functional analyses using different expression systems indicate that at least 50% reduction in NBCe1A activity would be required to induce severe pRTA [3,7,9]. However, no tight relationship between the degree of NBCe1A inactivation and the severity of acidemia exits, suggesting the involvement of other factors in the etiology of pRTA. Indeed, several mutants are found to display abnormal trafficking in mammalian cells [10,42,43]. As will be discussed later, defective membrane expression of NBCe1B in astrocytes may be responsible for the occurrence of migraine [10].

4. Physiological roles of NBCe1 in ocular homeostasis

The presence of NBCe1-like activity has been reported in several ocular tissues. Among these tissues, the physiological role of NBCe1 is established in the corneal endothelium. Thus, the corneal endothelium is known to mediate the electrogenic transport of sodium and bicarbonate into the aqueous humor, and this process is considered to be essential for corneal hydration and transparency [44]. Several lines of evidence suggest that NBCe1 is responsible for a majority of this transport. For example, Jentsch et al. found an electrogenic sodium-coupled bicarbonate cotransport activity compatible with NBCe1 in cultured bovine corneal endothelial cells [45]. Usui et al. later found the functional and molecular evidence for NBCe1 in cultured human corneal endothelial cells [46]. Immunohistological analysis confirmed the expression of NBCe1 in rat, human, and bovine corneal endothelium [12,13,47]. Furthermore, most of the pRTA patients with NBCe1 mutations presented with band keratopathy. The reduction of bicarbonate efflux by NBCe1 mutations may increase the local pH within the corneal stroma, which may facilitate local Ca$^{2+}$ deposition resulting in band keratopathy [13].

Immunohistological analysis also detected the expression of NBCe1 in rat and human lens epithelium [12,13]. Functional analysis in cultured human lens epithelial cells revealed the presence of Cl$^{-}$-independent, electrogenic Na$^+$-HCO$_3^-$ cotransporter activity. This transport
activity was largely suppressed by adenovirus-mediated transfer of a specific hammerhead ribozyme against NBCe1, consistent with a major role of NBCe1 in overall bicarbonate transport by the lens epithelium [13]. The lens is an avascular tissue, and the transport by lens epithelium may be essential for the maintenance of lens homeostasis and integrity [48]. A study in lens epithelial cell layers indeed detected an active fluid transport from their anterior to posterior sides against a hydrostatic pressure [49]. Probably, the transport activity of NBCe1 in lens epithelium may be essential for the lens homeostasis and transparency. Indeed, the pRTA patients with NBCe1 mutations often presented with cataracts.

Most of the pRTA patients with NBCe1 mutations also presented with glaucoma. Immunohistological analysis detected the expression of NBCe1 in human trabecular meshwork cells [13]. The electrogenic transport activity compatible with NBCe1 was also reported in human trabecular meshwork cells [50]. Because trabecular meshwork is the main site for aqueous outflow in the human eye [51], the inactivation of NBCe1 in trabecular meshwork cells may be responsible for the occurrence of high-tension glaucoma usually observed in the pRTA patients with homozygous NBCe1 mutations [10]. On the other hand, the NBCe1 expression was also detected in retina [12,52]. Interestingly, some of the family members carrying the heterozygous NBCe1 S982NfsX4 mutation, which has a dominant negative effect as will be discussed later, presented with normal-tension glaucoma without pRTA [10]. This type of glaucoma may be caused by dysregulation of extracellular pH in retina, because NBCe1 in retinal Müller cells may protect the excessive synaptic activities by counteracting the light-induced extracellular alkalosis [12,52,53].

NBCe1 was also found in human and rat pigmented and nonpigmented ciliary epithelial cells [12,13]. In addition to Na+/H+ and anion exchangers [54], NBCe1 may be also involved in influx and efflux of bicarbonate into/from these tissues, thereby contributing to the initial step of aqueous humor formation [55].

Regarding the NBCe1 variants expressed in ocular tissues, several studies suggest that NBCe1B is the predominant variant [12,47]. However, both NBCe1A and NBCe1B are indeed expressed in several ocular tissues [13,46]. Consistent with the latter view, the pRTA patient carrying the homozygous Q29X mutation, which inactivates NBCe1A but leaves NBCe1B and NBCe1C intact, presented with bilateral high-tension glaucoma [4]. She did not have band keratopathy or cataract.

5. NBCe1 mutations and migraine

It has been known that pH in the brain shows rapid changes in response to electrical activity. These changes in local pH may have an important influence on neurobiological responses by modifying numerous enzymes, ion channels, transporters, and receptors [19].

Among several acid/base transporters expressed in the brain, NBCe1 is intensively expressed in olfactory bulb, hippocampal dentate gyrus, and cerebellum, localizing in both glial cells and neurons [56]. Although a large number of transporters may be involved in the pH homeostasis
of the brain interstitial space, acid secretion by glial cells via inward electrogenic Na\(^+\)-HCO\(_3\)\(^-\) cotransporter NBCe1B may have a significant role in the prevention of excessive neural activities. In fact, alkalosis in extracellular spaces is generally associated with enhanced neuronal excitability, while acidosis is known to suppress neural activity [19]. A recent study using NBCe1 knockout (KO) mice confirmed that NBCe1 mediates a depolarization-induced alkanilization (DIA) response in astrocytes [57]. This study revealed that NBCe1 also contributes partially to a DIA response in hippocampal neurons [57]. Bevensee et al. initially reported that the expression of NBCe1B is more abundant in astrocytes than in neuron, while NBCe1C show the reverse pattern of expression [18]. However, the expression of NBCe1C was also found in rat astrocytes [22]. Despite the intensive expression of NBCe1 in brain and the potential contribution of NBCe1 to the extracellular pH regulation in brain, the physiological significance of NBCe1 in brain had still remained speculative. However, recent work revealed an unrecognized association of migraine with NBCe1 mutations [10].

Migraine is a common, disabling, multifactorial disorder, affecting more than 10% of the population with women more affected than men [58]. Although genetic factor plays a substantial role in ordinary migraine, the genetic basis has been established only in familial hemiplegic migraine (FHM), a rare autosomal dominant subtype of migraine with aura. In addition to a similar headache phase as found in ordinarily migraine, FHM patients experience prolonged hemiparesis [59]. Thus far, three genes have been identified as the genetic basis for FHM: CACNA1A encoding the \(\alpha_1\) subunit of voltage-gated neuronal Cav2.1 calcium channels [60], ATP1A2 encoding the \(\alpha_2\) subunit of Na\(^+\)/K\(^+\) ATPase [61], and SCN1A encoding the neuronal voltage-gated sodium channel Nav1.1 [62]. These mutations are thought to cause migraine by enhancing neuronal excitability [63].

We recently identified two sisters with pRTA, ocular abnormalities and hemiplegic migraine. Genetic analysis excluded pathological mutation in CACNA1A, ATP1A2, and SCN1A, but identified the homozygous S982NfsX4 mutation in the C-terminus of NBCe1 [10]. Several heterozygous members of the family also presented with glaucoma and migraine with or without aura. This mutant showed a normal electrogenic activity in Xenopus oocytes. When expressed in mammalian cells, however, the S982NfsX4 mutant showed almost no transport activity due to a predominant retention in the endoplasmic reticulum (ER). Several mutant proteins that are retained in the ER are known to exert a dominant negative effect by forming hetero-oligomer complexes with wild-type proteins [64], and NBCe1 can also form the oligomer complexes [65]. Indeed, co-expression analysis uncovered a dominant negative effect of the mutant through hetero-oligomer formation with wild-type NBCe1, which may be responsible for the occurrence of migraine and glaucoma in the heterozygous family members.

To further substantiate NBCe1 mutations as a cause of migraine, we re-investigated the other pRTA pedigrees with distinct NBCe1 mutations, and found 4 additional homozygous patients with migraine: hemiplegic migraine with episodic ataxia in L522P [8], migraine with aura in N721TfsX29 [6], and migraine without aura in R510H and R881C [3,7]. Transient expression of GFP-tagged NBCe1B constructs carrying these mutations in C6 glioma cells revealed a remarkable coincidence between the apparent lack of membrane expression and the occurrence of migraine. From these and other results, we concluded that the near total loss of
NBCe1B activity in astrocytes can cause migraine potentially through dysregulation of synaptic pH [10]. We cannot exclude a possibility that the inactivation of NBCe1C is also involved in the pathogenesis of migraine.

Cerebral cortical hyperexcitability causing cortical spreading depression (CSD) seems to be the underlying pathophysiological mechanism of migraine aura [63]. In general, neuronal firing may lead to a rise in extracellular K⁺ concentration and further depolarization, but uptake of K⁺ into astrocytes can counteract this process. Therefore, enhanced neurotransmitter release by \textit{CACNA1A} mutations, excessive neuronal firing by \textit{SCN1A} mutations, or impaired clearance of K⁺ and/or glutamate by \textit{ATP1A2} mutations can all induce CSD [63]. Neuronal excitation may also elicit an initial extracellular alkalosis, probably mediated by Ca²⁺/H⁺ exchange [19]. Upon depolarization, however, glial cells secret acid via inward electrogenic Na⁺-HCO₃⁻ cotransport NBCe1, i.e. DIA, overwhelming the initial extracellular alkalosis. Under normal condition, the net extracellular acidosis due to DIA makes surrounding neuronal cells less excitable, because protons suppress excitatory NMDA receptors, with a steep sensitivity in the physiological range of extracellular [19]. Absence of DIA due to defective membrane expression of NBCe1 in astrocytes may cause a positive feedback loop of increased neuronal activity leading to further NMDA-mediated neuronal hyperactivity, causing complete depolarization of a sizable population of brain cells, i.e. CSD. We therefore think that migraine associated with NBCe1 mutations represents a primary headache most likely caused by dysfunctional local pH regulation in the brain as shown in Figure 3.

**Figure 3.** Migraine-associated transporters. While \textit{SCN1A} and \textit{CACNA1A} may directly regulate neuron excitation, \textit{ATP1A2} may regulate neuron excitation indirectly via uptake of K⁺ and/or glutamate into astrocytes. On the other hand, NBCe1-mediated uptake of HCO₃⁻ into astrocytes may also regulate neuron excitation by affecting pH-sensitive NMDA receptors.
6. Roles of N-terminal sequences in NBCe1 functions

When expressed in *Xenopus* oocytes, NBCe1B and NBCe1C showed much lower activities than that of NBCe1A [66-68]. The deletion from of the cytoplasmic N-terminus of an 87-amino acid sequence markedly enhanced the activities of both NBCe1B and NBCe1C by more than 3-fold, indicating that this sequence contains an autoinhibitory domain [66,68]. On the other hand, this sequence also contains a binding domain for inositol 1,4,5-trisphosphate receptors (IP3R) binding protein released with IP3 (IRBIT). IRBIT is dissociated from IP3R in the presence of physiological concentrations of IP3, the process of which has an important role in the regulation of IP3R functions [69,70].

We and others found that IRBIT binds to and activates NBCe1B and NBCe1C expressed in *Xenopus* oocytes [67,71]. Because this binding requires the cytoplasmic sequence of a 62-amino acid sequence in the N-terminus of NBCe1B and NBCe1C, IRBIT does not bind to NBCe1A that lacks this sequence [67]. Co-expression of IRBIT markedly activates the NBCe1B activity by several-fold. Because this stimulation is not associated with the significant changes in the amount of NBCe1B expressed in the plasma membranes of *Xenopus* oocytes, IRBIT may induce the stimulation of per-molecule activity of NBCe1B [66,68]. Interestingly, Lee et al. found that a mutant IRBIT lacking a protein phosphatase-1 (PP-1) binding site stimulates NBCe1B to a 50% greater than can be achieved by the removal of autoinhibitory domain [68]. These results suggest that the stimulatory mechanism of IRBIT may involve not only the neutralization of autoinhibitory domain but also other factors.

The stimulation of NBCe1B by IRBIT has been also confirmed in pancreatic ducts *in vivo* [25]. Thus in secretory epithelia such as pancreatic ducts, IRBIT has a central role in fluid and bicarbonate secretion by activating both NBCe1B and the cystic fibrosis transmembrane conductance regulator CFTR [25]. The subsequent study revealed that the with-no-lysine (WNK) kinases act as scaffolds to recruit Ste20-related proline/alanine-rich kinase (SPAK), which phosphorylates CFTR and NBCe1B, reducing their surface expression. In addition to the direct activation of NBCe1B and CFTR, IRBIT opposed the effects of WNKs and SPAK by recruiting PP-1 to dephosphorylate CFTR and NBCe1B, restoring their surface expression [72]. In contrast to these complex modes of IRBIT-mediated transport stimulation in secretory epithelia, the dephosphorylation of IRBIT by PP-1 may rather partially suppress the stimulatory effect of IRBIT on NBCe1B in *Xenopus* oocytes, which do not express WNKs or SPAK [68,73].

The injection of inositol 4,5-bisphosphate (PIP2) into *Xenopus* oocytes stimulated the whole currents of NBCe1B and NBCe1C [74]. IRBIT reduced the PIP2-induced stimulation of NBCe1B and NBCe1C, suggesting that IRBIT and PIP2 may compete with one another in stimulating NBCe1B and NBCe1C [71]. In addition to the regulation by the binding of IRBIT or PIP2, the N-terminus of NBCe1B and NBCe1C may also play a role in the inhibition by intracellular Mg2+ [75].

7. Phenotypes of NBCe1-deficient mice

Two types of NBCe1-deficient mice, NBCe1 KO and W516X knockin (KI) mice, have been produced [11,14]. Both types of mice show severe acidosis and early lethality. Thus, NBCe1
KO mice exhibited severe metabolic acidosis (blood HCO$_3^-$ concentration of 5.3 mM), growth retardation, hyperaldosteronism, anemia and splenomegaly, abnormal enamel mineralization, intestinal obstruction, and early death before weaning. Splenomegaly might be due to hemolytic anemia due to severe acidemia. The white pulp and the red pulp were severely disrupted in spleen of KO mice. A significant reduction in the cAMP-stimulated short circuit current was detected in colon of KO mice in the presence of a carbonic anhydrase inhibitor acetazolamide, which might reduce the availability of HCO$_3^-$.

A homozygous NBCe1 W516X mutation was identified in a girl with severe pRTA (blood HCO$_3^-$ concentration of 10 mM), growth retardation, and the typical ocular abnormalities including band keratopathy, cataracts, and glaucoma [11]. Homozygous W516X KI mice also presented with severe metabolic acidosis (blood HCO$_3^-$ concentration of 3.9 mM), growth retardation, hyperaldosteronism, anemia and splenomegaly, and early death before weaning [11]. Due to the process of nonsense-mediated decay, the expression of NBCe1 mRNA was halved in the heterozygous and virtually absent in the homozygous W516X KI mice. The NBCe1 activity in isolated renal proximal tubules from the homozygous KI mice was severely reduced to less than 20% of the activity in tubules from wild-type mice. The rate of bicarbonate absorption in the homozygous KI mice was also markedly reduced to less than 20% of that in wild-type mice, confirming the indispensable role of NBCe1 in bicarbonate absorption from renal proximal tubules. Alkali therapy was effective in prolonging the survival, and partially improving growth retardation and bone abnormalities of the homozygous KI mice. The prolonged survival time by alkali therapy uncovered the development of corneal opacities due to corneal edema in the homozygous KI mice. These results confirmed that the normal NBCe1 activity in corneal endothelium is essential for the maintenance of corneal transparency not only in humans but also in mice [11].

Unlike NBCe1 KO and W516X KI mice, NHE3 KO mice showed only a mild acidemia with blood HCO$_3^-$ level of around 21 mM [76]. In the apical membranes of renal proximal tubules, Na$^+$/H$^+$ exchanger type 3 (NHE3) has been considered to mediate a majority of proton secretion into lumen [77]. However, functional analysis using isolated renal proximal tubules from NHE3 KO mice revealed the residual amiloride-sensitive NHE activity, which corresponded to approximately 50% of the wild-type activity [78]. This residual NHE activity, which could represent NHE8 [79], might be able to at least partially compensate for the loss of NHE3 activity. In contrast to such an effective compensation mechanism in the apical membranes, Na$^+$-dependent and Na$^+$-independent Cl$^-$/$HCO$_3^-$ exchangers in the basolateral membranes of renal proximal tubules [35,36] may be unable to compensate for the loss of NBCe1A activity.

**Author details**

George Seki, Shoko Horita, Masashi Suzuki, Osamu Yamazaki and Hideomi Yamada  
Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Japan
8. References

[1] Romero MF, Hediger MA, Boulpaep EL, Boron WF. Expression cloning and characterization of a renal electrogenic Na+/HCO₃⁻ cotransporter. Nature 1997; 387: 409-413.

[2] Romero MF, Boron WF. Electrogenic Na⁺/HCO₃⁻ cotransporters: cloning and physiology. Annu Rev Physiol 1999; 61: 699-723.

[3] Igarashi T, Inatomi J, Sekine T, et al. Mutations in SLC4A4 cause permanent isolated proximal renal tubular acidosis with ocular abnormalities. Nat Genet 1999; 23: 264-266.

[4] Igarashi T, Inatomi J, Sekine T, et al. Novel nonsense mutation in the Na⁺/HCO₃⁻ cotransporter gene (SLC4A4) in a patient with permanent isolated proximal renal tubular acidosis and bilateral glaucoma. J Am Soc Nephrol 2001; 12: 713-718.

[5] Dinour D, Chang MH, Satoh J, et al. A novel missense mutation in the sodium bicarbonate cotransporter (NBCe1/SLC4A4) causes proximal tubular acidosis and glaucoma through ion transport defects. J Biol Chem 2004; 279: 52238-52246.

[6] Inatomi J, Horita S, Braverman N, et al. Mutational and functional analysis of SLC4A4 in a patient with proximal renal tubular acidosis. Pflugers Arch 2004; 448: 438-444.

[7] Horita S, Yamada H, Inatomi J, et al. Functional analysis of NBC1 mutants associated with proximal renal tubular acidosis and ocular abnormalities. J Am Soc Nephrol 2005; 16: 2270-2278.

[8] Demirci FY, Chang MH, Mah TS, Romero MF, Gorin MB. Proximal renal tubular acidosis and ocular pathology: a novel missense mutation in the gene (SLC4A4) for sodium bicarbonate cotransporter protein (NBCe1). Mol Vis 2006; 12: 324-330.

[9] Suzuki M, Vaisbich MH, Yamada H, et al. Functional analysis of a novel missense NBC1 mutation and of other mutations causing proximal renal tubular acidosis. Pflugers Arch 2008; 455: 583-593.

[10] Suzuki M, Van Paesschen W, Stalmans I, et al. Defective membrane expression of the Na⁺-HCO₃⁻ cotransporter NBCe1 is associated with familial migraine. Proc Natl Acad Sci U S A 2010; 107: 15963-15968.

[11] Lo YF, Yang SS, Seki G, et al. Severe metabolic acidosis causes early lethality in NBC1 W516X knock-in mice as a model of human isolated proximal renal tubular acidosis. Kidney Int 2011; 79: 730-741.

[12] Bok D, Schibler MJ, Pushkin A, et al. Immunolocalization of electrogenic sodium-bicarbonate cotransporters pNBC1 and kNBC1 in the rat eye. Am J Physiol Renal Physiol 2001; 281: F920-F935.

[13] Usui T, Hara M, Satoh H, et al. Molecular basis of ocular abnormalities associated with proximal renal tubular acidosis. J Clin Invest 2001; 108: 107-115.

[14] Gawenis LR, Bradford EM, Prasad V, et al. Colonic anion secretory defects and metabolic acidosis in mice lacking the NBC1 Na⁺/HCO₃⁻ cotransporter. J Biol Chem 2007; 282: 9042-9052.

[15] Boron WF, Chen L, Parker MD. Modular structure of sodium-coupled bicarbonate transporters. J Exp Biol 2009; 212: 1697-1706.
[16] Liu Y, Xu JY, Wang DK, Wang L, Chen LM. Cloning and identification of two novel NBCe1 splice variants from mouse reproductive tract tissues: a comparative study of NBCT genes. Genomics 2011; 98: 112-119.

[17] Abuladze N, Song M, Pushkin A, et al. Structural organization of the human NBC1 gene: kNBC1 is transcribed from an alternative promoter in intron 3. Gene 2000; 251: 109-122.

[18] Bevensee MO, Schmitt BM, Choi I, Romero MF, Boron WF. An electrogenic Na⁺-HCO₃⁻ cotransporter (NBC) with a novel COOH-terminus, cloned from rat brain. Am J Physiol Cell Physiol 2000; 278: C1200-C1211.

[19] Chesler M. Regulation and modulation of pH in the brain. Physiol Rev 2003; 83: 1183-1221.

[20] Marino CR, Jeanes V, Boron WF, Schmitt BM. Expression and distribution of the Na⁺-HCO₃⁻ cotransporter in human pancreas. Am J Physiol 1999; 277: G487-G494.

[21] Satoh H, Moriyama N, Hara C, et al. Localization of Na⁺-HCO₃⁻ cotransporter (NBC-1) variants in rat and human pancreas. Am J Physiol Cell Physiol 2003; 284: C729-C737.

[22] Majumdar D, Maunsbach AB, Shacka JJ, et al. Localization of electrogenic Na/bicarbonate cotransporter NBCe1 variants in rat brain. Neuroscience 2008; 155: 818-832.

[23] Ishiguro H, Steward MC, Lindsay AR, Case RM. Accumulation of intracellular HCO₃⁻ by Na⁺-HCO₃⁻ cotransport in interlobular ducts from guinea-pig pancreas. J Physiol 1996; 495 (Pt 1): 169-178.

[24] Ishiguro H, Steward MC, Wilson RW, Case RM. Bicarbonate secretion in interlobular ducts from guinea-pig pancreas. J Physiol 1996; 495 (Pt 1): 179-191.

[25] Yang D, Shcheynikov N, Zeng W, et al. IRBIT coordinates epithelial fluid and HCO₃⁻ secretion by stimulating the transporters pNBC1 and CFTR in the murine pancreatic duct. J Clin Invest 2009; 119: 193-202.

[26] Steward MC, Ishiguro H, Case RM. Mechanisms of bicarbonate secretion in the pancreatic duct. Annu Rev Physiol 2005; 67: 377-409.

[27] Boron WF. Acid-base transport by the renal proximal tubule. J Am Soc Nephrol 2006; 17: 2368-2382.

[28] Yoshitomi K, Burckhardt BC, Fromter E. Rheogenic sodium-bicarbonate cotransport in the peritubular cell membrane of rat renal proximal tubule. Pflugers Arch 1985; 405: 360-366.

[29] Seki G, Coppola S, Fromter E. The Na⁺-HCO₃⁻ cotransporter operates with a coupling ratio of 2 HCO₃⁻ to 1 Na⁺ in isolated rabbit renal proximal tubule. Pflugers Arch 1993; 425: 409-416.

[30] Seki G, Coppola S, Yoshitomi K, et al. On the mechanism of bicarbonate exit from renal proximal tubular cells. Kidney Int 1996; 49: 1671-1677.

[31] Muller-Berger S, Nesterov VV, Fromter E. Partial recovery of in vivo function by improved incubation conditions of isolated renal proximal tubule. II. Change of Na-HCO₃⁻ cotransport stoichiometry and of response to acetazolamide. Pflugers Arch 1997; 434: 383-391.
[32] Muller-Berger S, Ducoudret O, Diakov A, Fromter E. The renal Na-HCO₃-cotransporter expressed in Xenopus laevis oocytes: change in stoichiometry in response to elevation of cytosolic Ca²⁺ concentration. Pflugers Arch 2001; 442: 718-728.

[33] Gross E, Hawkins K, Abuladze N, et al. The stoichiometry of the electrogenic sodium bicarbonate cotransporter NBC1 is cell-type dependent. J Physiol 2001; 531: 597-603.

[34] Chen LM, Liu Y, Boron WF. Role of an extracellular loop in determining the stoichiometry of Na⁺-HCO₃ cotransporters. J Physiol 2011; 589: 877-890.

[35] Preisig PA, Alpern RJ. Basolateral membrane H-OH-HCO₃ transport in the proximal tubule. Am J Physiol 1989; 256: F751-F765.

[36] Seki G, Fromter E. Acetazolamide inhibition of basolateral base exit in rabbit renal proximal tubule S2 segment. Pflugers Arch 1992; 422: 60-65.

[37] Stehberger PA, Shmuukler BE, Stuart-Tilley AK, Peters LL, Alper SL, Wagner CA. Distal renal tubular acidosis in mice lacking the AE1 (band3) Cl⁻/HCO₃⁻ exchanger (slc4a1). J Am Soc Nephrol 2007; 18: 1408-1418.

[38] Zhu Q, Kao L, Azimov R, et al. Topological location and structural importance of the NBCe1-A residues mutated in proximal renal tubular acidosis. J Biol Chem 2010; 285: 13416-13426.

[39] Chang MH, DiPiero J, Sonnichsen FD, Romero MF. Entry to "formula tunnel" revealed by SLC4A4 human mutation and structural model. J Biol Chem 2008; 283: 18402-18410.

[40] Deda G, Ekim M, Guven A, Karagol U, Tumer N. Hypopotassemic paralysis: a rare presentation of proximal renal tubular acidosis. J Child Neurol 2001; 16: 770-771.

[41] Parker MD, Qin X, Williamson RC, Toye AM, Boron WF. HCO₃⁻-independent conductance with a mutant Na/HCO₃ cotransporter (SLC4A4) in a case of proximal renal tubular acidosis with hypokalemic paralysis. J Physiol 2012: (in press) doi: 10.1113/jphysiol.2011.224733

[42] Li HC, Szigligeti P, Worrell RT, Matthews JB, Conforti L, Soleimani M. Missense mutations in Na⁺:HCO₃⁻ cotransporter NBC1 show abnormal trafficking in polarized kidney cells: a basis of proximal renal tubular acidosis. Am J Physiol Renal Physiol 2005; 289: F61-F71.

[43] Toye AM, Parker MD, Daly CM, et al. The human NBCe1-A mutant R881C, associated with proximal renal tubular acidosis, retains function but is mistargeted in polarized renal epithelia. Am J Physiol Cell Physiol 2006; 291: C788-C801.

[44] Hodson S, Miller F. The bicarbonate ion pump in the endothelium which regulates the hydration of rabbit cornea. J Physiol 1976; 263: 563-577.

[45] Jentsch TJ, Keller SK, Koch M, Wiederholt M. Evidence for coupled transport of bicarbonate and sodium in cultured bovine corneal endothelial cells. J Membr Biol 1984; 81: 189-204.

[46] Usui T, Seki G, Amano S, et al. Functional and molecular evidence for Na⁺-HCO₃ cotransporter in human corneal endothelial cells. Pflugers Arch 1999; 438: 458-462.

[47] Sun XC, Bonanno JA, Jelamskii S, Xie Q. Expression and localization of Na⁺-HCO₃ cotransporter in bovine corneal endothelium. Am J Physiol Cell Physiol 2000; 279: C1648-C1655.
[48] Mathias RT, Rae JL, Baldo GJ. Physiological properties of the normal lens. Physiol Rev 1997; 77: 21-50.
[49] Fischbarg J, Diecke FP, Kuang K, et al. Transport of fluid by lens epithelium. Am J Physiol 1999; 276: C548-C557.
[50] Lepple-Wienhues A, Rauch R, Clark AF, Grassmann A, Berweck S, Wiederholt M. Electrophysiological properties of cultured human trabecular meshwork cells. Exp Eye Res 1994; 59: 305-311.
[51] Bill A. Blood circulation and fluid dynamics in the eye. Physiol Rev 1975; 55: 383-417.
[52] Newman EA. Sodium-bicarbonate cotransport in retinal astrocytes and Muller cells of the rat. Glia 1999; 26: 302-308.
[53] Borgula GA, Karwoski CJ, Steinberg RH. Light-evoked changes in extracellular pH in frog retina. Vision Res 1989; 29: 1069-1077.
[54] Counillon L, Touret N, Bidet M, et al. Na+/H+ and Cl-/HCO3- antiporters of bovine pigmented ciliary epithelial cells. Pflugers Arch 2000; 440: 667-678.
[55] Shahidullah M, To CH, Pelis RM, Delamere NA. Studies on bicarbonate transporters and carbonic anhydrase in porcine nonpigmented ciliary epithelium. Invest Ophthalmol Vis Sci 2009; 50: 1791-1800.
[56] Schmitt BM, Berger UV, Douglas RM, et al. Na/HCO3 cotransporters in rat brain: expression in glia, neurons, and choroid plexus. J Neurosci 2000; 20: 6839-6848.
[57] Svichar N, Esquenazi S, Chen HY, Chesler M. Preemptive regulation of intracellular pH in hippocampal neurons by a dual mechanism of depolarization-induced alkalinization. J Neurosci 2011; 31: 6997-7004.
[58] Lipton RB, Scher AI, Kolodner K, Liberman J, Steiner TJ, Stewart WF. Migraine in the United States: epidemiology and patterns of health care use. Neurology 2002; 58: 885-894.
[59] The International Classification of Headache Disorders: 2nd edition. Cephalalgia 2004; 24 Suppl 1: 9-160.
[60] Ophoff RA, Terwindt GM, Vergouwe MN, et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell 1996; 87: 543-552.
[61] De Fusco M, Marconi R, Silvestri L, et al. Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat Genet 2003; 33: 192-196.
[62] Dichgans M, Freilinger T, Eckstein G, et al. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet 2005; 366: 371-377.
[63] Goadsby PJ. Recent advances in understanding migraine mechanisms, molecules and therapeutics. Trends Mol Med 2007; 13: 39-44.
[64] Alper SL. Genetic diseases of acid-base transporters. Annu Rev Physiol 2002; 64: 899-923.
[65] Kao L, Sassani P, Azimov R, et al. Oligomeric structure and minimal functional unit of the electrogenic sodium bicarbonate cotransporter NBCe1-A. J Biol Chem 2008; 283: 26782-26794.
[66] McAlear SD, Liu X, Williams JB, McNicholas-Bevensee CM, Bevensee MO. Electrogenic Na/HCO₃ cotransporter (NBCe1) variants expressed in Xenopus oocytes: functional comparison and roles of the amino and carboxy termini. J Gen Physiol 2006; 127: 639-658.

[67] Shirakabe K, Priori G, Yamada H, et al. IRBIT, an inositol 1,4,5-trisphosphate receptor-binding protein, specifically binds to and activates pancreas-type Na⁺/HCO₃ cotransporter 1 (pNBC1). Proc Natl Acad Sci U S A 2006; 103: 9542-9547.

[68] Lee SK, Boron WF, Parker MD. Relief of autoinhibition of the electrogenic Na-HCO₃ cotransporter NBCe1-B: role of IRBIT vs. amino-terminal truncation. Am J Physiol Cell Physiol 2012; 302: C518-C526.

[69] Ando H, Mizutani A, Matsu-ura T, Mikoshiba K. IRBIT, a novel inositol 1,4,5-trisphosphate (IP3) receptor-binding protein, is released from the IP3 receptor upon IP3 binding to the receptor. J Biol Chem 2003; 278: 10602-10612.

[70] Ando H, Mizutani A, Kiefer H, Tsuzurugi D, Michikawa T, Mikoshiba K. IRBIT suppresses IP3 receptor activity by competing with IP3 for the common binding site on the IP3 receptor. Mol Cell 2006; 22: 795-806.

[71] Thornell IM, Wu J, Bevensee MO. The IP3 receptor-binding protein IRBIT reduces phosphatidylinositol 4,5-bisphosphate (PIP2) stimulation on of Na/bicarbonate cotransporter NBCe1 variants expressed in Xenopus laevis oocytes (Abstract). FASEB J 2010; 24: 815.816.

[72] Yang D, Li Q, So I, et al. IRBIT governs epithelial secretion in mice by antagonizing the WNK/SPAK kinase pathway. J Clin Invest 2011; 121: 956-965.

[73] Devogelaere B, Beullens M, Sammels E, et al. Protein phosphatase-1 is a novel regulator of the interaction between IRBIT and the inositol 1,4,5-trisphosphate receptor. Biochem J 2007; 407: 303-311.

[74] Wu J, McNicholas CM, Bevensee MO. Phosphatidylinositol 4,5-bisphosphate (PIP2) stimulates the electrogenic Na/HCO₃ cotransporter NBCe1-A expressed in Xenopus oocytes. Proc Natl Acad Sci U S A 2009; 106: 14150-14155.

[75] Yamaguchi S, Ishikawa T. The electrogenic Na⁺-HCO₃ cotransporter NBCe1-B is regulated by intracellular Mg²⁺. Biochem Biophys Res Commun 2008; 376: 100-104.

[76] Schultheis PJ, Clarke LL, Meneton P, et al. Renal and intestinal absorptive defects in mice lacking the NHE3 Na⁺/H⁺ exchanger. Nat Genet 1998; 19: 282-285.

[77] Alpern RJ. Cell mechanisms of proximal tubule acidification. Physiol Rev 1990; 70: 79-114.

[78] Choi JY, Shah M, Lee MG, et al. Novel amiloride-sensitive sodium-dependent proton secretion in the mouse proximal convoluted tubule. J Clin Invest 2000; 105: 1141-1146.

[79] Goyal S, Vanden Heuvel G, Aronson PS. Renal expression of novel Na⁺/H⁺ exchanger isoform NHE8. Am J Physiol Renal Physiol 2003; 284: F467-F473.