The isoform-specific deletion of a bicarbonate-transporting membrane protein in mice

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ABSTRACT

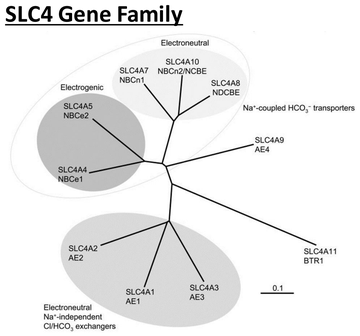
Loss of the sodium/bicarbonate cotransporter NBCe1 results in low blood pH (acidosis) and poor dentition. NBCe1 encodes two isoforms: NBCe1-A and NBCe1-B. NBCe1-A loss directly causes acidosis. We generated a novel strain of mouse predicted to lack only NBCe1-B to investigate whether poor dentition follows acidosis or follows NBCe1-B loss from enamel-secreting cells.
The abundance of NBCe1-A (relative to actin) was probed by western blot of kidney lysates and quantified by densitometry. Blood pH and teeth of knockout mice were assessed by blood gas analysis and microscopy. The results showed that, compared to wild-type mice, knockout mice have [1] a normal abundance of NBCe1-A, [2] normal blood pH and [3] unusually eroded chalky-white teeth. Thus, knockout mice are suitable for this study and the brittle teeth are a direct result of NBCe1-B loss. Enamel defect cannot be treated by alkali therapy and alternative treatment is required.

PURPOSE

The purpose of this study was to clarify that the dental abnormalities are a direct result of NBCe1-B loss from enamel cells

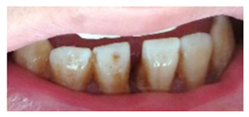
INTRODUCTION

SLC4 Gene Family



The SLC4 gene family includes ten membrane proteins, eight of which encode bicarbonate transporters involved in intracellular pH (pH_i) regulation[1] .

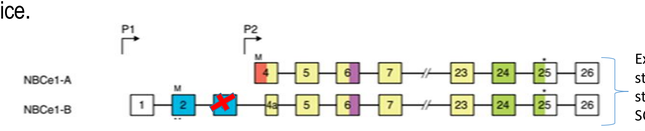
Loss of NBCe1 results in a rare autosomal recessive disease named proximal renal tubular acidosis (pRTA) characterized by acidic blood and dental abnormalities (easily-worn tooth enamel).



Poor dentition in a patient with pRTA [2]

NBCe1 acts to maintain intracellular and plasma pH [2]
NBCe1-A is expressed in the kidneys and regulates blood pH by claiming HCO_3^- from filtered blood plasma in kidney tubules.
NBCe1-B is expressed in a wide variety of non renal-cells.

The structure of the NBCe1 gene allows us to selectively disrupt NBCe1-B in mice.



Exon structure of structure of SCL4A4 gene products

EXPERIMENTAL METHODS & RESULTS

Blood arterial gas analysis was performed to evaluate and compare the blood composition of wild-type and knockout mice (see Figure 1).

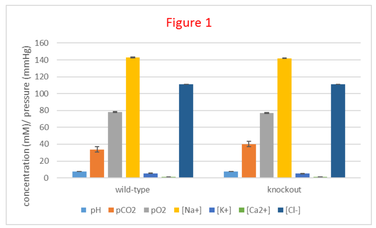
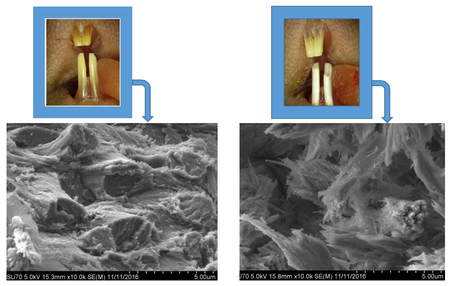


Figure 1 shows the blood gas composition as well as the blood chemistry of wild-type and Knockout mice. Even though, the electrogenic sodium bicarbonate cotransporter (NBCe1-B) gene has been lost in Knockout mice, their blood gas composition as well as blood chemistry were relatively unperturbed.
Loss of NBCe1-B has neither affected the blood pH nor perturbed the distribution of the electrolytes.

Tooth structure from wild-type and knockout mice was analyzed using scanning electron microscopy (see Figure 2).



Wild-type mouse Knockout mouse

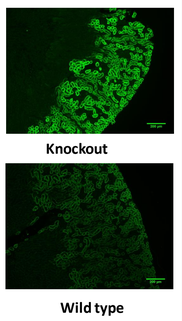
Figure 2

Figure 2 represents SEM images of tooth structure from wild-type and knockout mice. Figure 2 shows :
• wild-type mice have well-defined and organized tooth enamel.
• knockout mice have dispersed and non uniform tooth enamel.

NBCe1 presence from wild-type and knockout kidneys (see Figure 3)

NBCe1 protein in wild-type and knockout kidneys was immunolocalized. The green fluorescence shows the presence of NBCe1-A on the basolateral side of the membrane in both wild-type and knockout kidneys. Both images reveal a similar pattern in the expression of NBCe1 suggesting that distribution of NBCe1-A is unaffected by NBCe1-B deletion.

Figure 3: Kidney staining



Knockout

Wild type

Kidney lysates from wild type and knockout mice were prepared. The amount of protein from each kidney sample was measured using bicinchonic Acid assay (BCA assay) to estimate the amount of proteins that will be loaded on Nupage protein gels.

Kidney lysates were separated by electrophoresis on polyacrylamide gels

NBCe1-A protein from wild-type and knockout kidney samples was probed and its abundance assessed by densitometry (see Figure 4 &6).

The white fluorescent bands with a molecular weight between 171 and 117 kDA represent the NBCe1-A protein found in the wild-type and knockout kidneys. The western blot

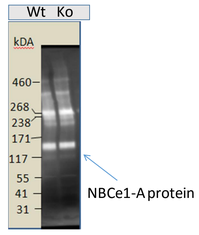


Figure 4 (on the left) illustrates NBCe1-A abundance present in kidney samples obtained from wild-type and knockout mice

The actin abundance from each sample was also quantified by densitometry (see Figure 6 &5) to standardize and normalize the abundance of NBCe1-A from each sample.

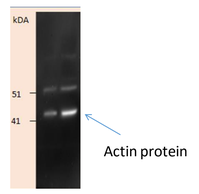


Figure 5 (on the left)
Illustrates the Actin abundance present in kidney samples from wild-type and knockout mice.

We Normalize the amount of NBCe1 loaded from the wild-type and knockout mice. We then performed paired t-test to see whether the amount of NBCe1-A in knockout mice is statistically different from the amount in NBCe1-A present in wild-type mice(see Figure 6).

a paired t-test shows that the two bars in figure 7 are not significantly different. Our null hypothesis was validated.

null hypothesis= no difference in NBCe1-A.

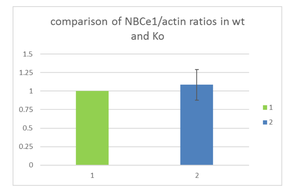


Figure 6

LEGEND
Green=wild-type
Blue=Knockout

SUMMARY AND SIGNIFICANCE

The study of NBCe1-B knockout mice has shown that:
• Loss of NBCe1-B does not affect NBCe1-A expression in the kidney.
• Blood pH and blood chemistry are unaffected by NBCe1-B deletion.
• The weakening of tooth enamel is a consequence of NBCe1-B loss.

NBCe1-B mice constitute a suitable model for the study of pRTA-associated dental abnormalities signs. We conclude that dental abnormalities in pRTA are a direct result from NBCe1-B loss and not a secondary effect of acidosis. Thus the correction of this defect cannot be addressed by alkali therapy . New drugs need to be developed to restore NBCe1 action to enamel secreting cells.

FUTURE WORK

We will investigate drugs that can directly target and restore NBCe1 action to enamel secreting cells.

REFERENCES

[1]Parker and Boron, Physiology Rev 2013, 93: 803-959
[2] Myers et al. A novel mutant Na^+/HCO_3^- cotransporter NBCe1 in a case of compound-heterozygous inheritance of proximal renal tubular acidosis. J Physiol 594 (2016) pp 6267–6286.