Molecular Characterization Of A Novel Mutation In The Renal Nacl Cotransporter Causing Gitelman´s Syndrome By Impairing Transporter Trafficking

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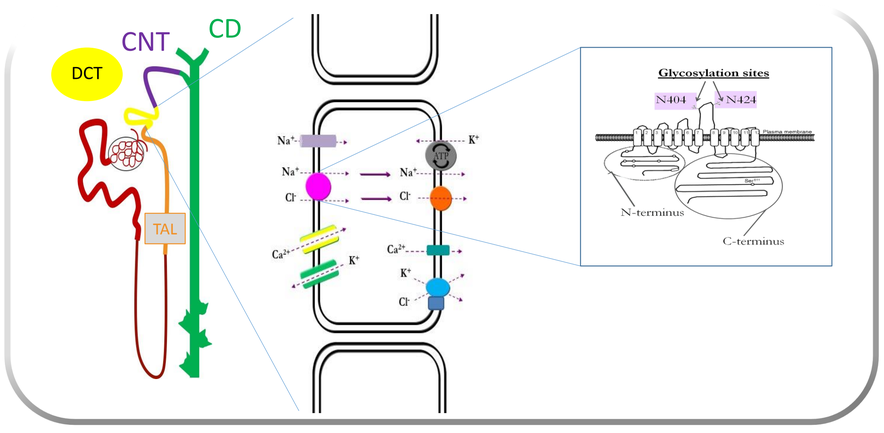
Gitelman's Syndrome

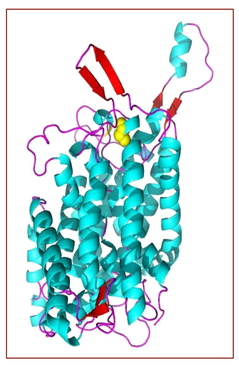
- Rare autosomal recessive disease characterized by electrolytic alterations
- Gitelman's electrolyte abnormalities are similar to those induced by treatment with Thiazide diuretics or other drugs that inhibit the Na-Cl cotransporter in the distal convoluted tubule (DCT) of the kidney
- Impaired Na^+ absorption causing **hypovolemia and therefore low BP.**
- Renal volume receptors govern sodium balance via the renin-angiotensin-aldosterone system (**marked RAAS stimulation**)
- Aldosterone stimulates ENaC and this leads to loss of K^+ (**hypokalemia**)
- Hypovolemia leads to increased Ca^{2+} reabsorption in the proximal tubule (**hypocalciuria**)
- Mg^{2+} loss (**hypomagnesemia**) due to the loss of early DCT which has the TRPM6 channels

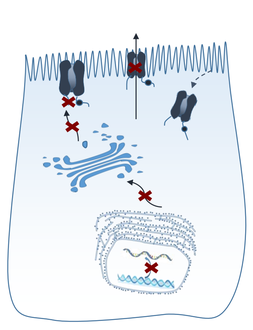
Distal Convoluted Tubule



Novel Mutation

DNA **c.1204G>A**
PROT **p.Gly394Asp**
missense





Mutations can lead to
- impaired synthesis of the protein,
- impaired trafficking of the protein to the cell surface,
- impaired functions of the protein at the cell surafce,
- impaired activation of the protein at the cell surface,
- enhanced degradation of the protein

Aim

Over 100 mutations are known, but the molecular mechanisms underlying the impaired function remained unclear.
In our cohort of GS a young woman presented with a NCC point mutation (c.1204G>A) never reported before, which causes an aminoacid exchange (Gly394Asp). Therefore, we used a molecular biology approach to investigate how this mutation affected NCC functionality.

Index Case

Woman

Age 37

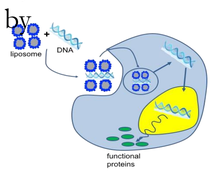
- K^+: 2.5 mEq/L; *(Norm: ~4.0 mEq/L)*
- Mg^{2+}: 0.60 mmol/L, *(Norm: ~0.90 mmol/L)*
- Ca^{2+}: 1.35mmol/L; *(Norm: ~1.19-1.29 mmol/L)*
- Aldosterone: 0.75 nmol/L; *(Norm: ~0.30 nmol/L)*
- BP: 110/68 mmHg; *(Norm: ~120/80 mmHg)*

Diagnosis:

GITELMAN'S SYNDROME

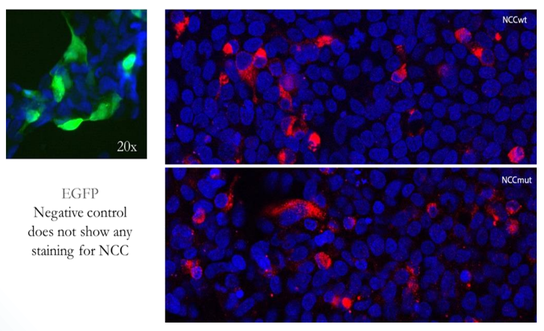
Material and Methods

We created different expression vectors containing either the wild type or the mutated NCC (SLC12A3) coding sequence. The DNA was then transfected into HEK293 cells and RNA into Xenopus laevis oocytes. We then assessed the expression, maturation, and the trafficking of the protein by western blot, immunohistochemistry (IHC) with confocal microscopy, and the NCC functionality by Na^{22+} uptake

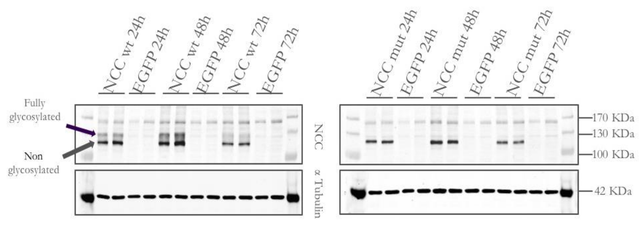


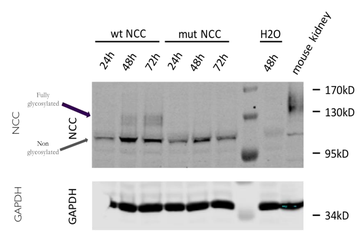
Results

HEK293 cell line

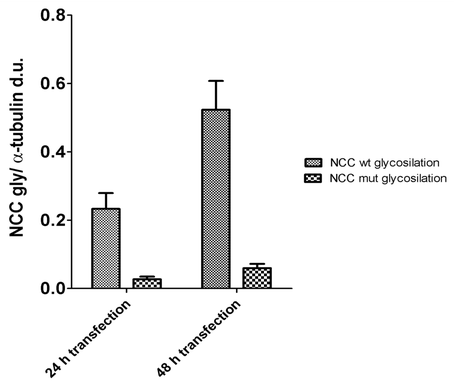


EGFP
Negative control
does not show any staining for NCC

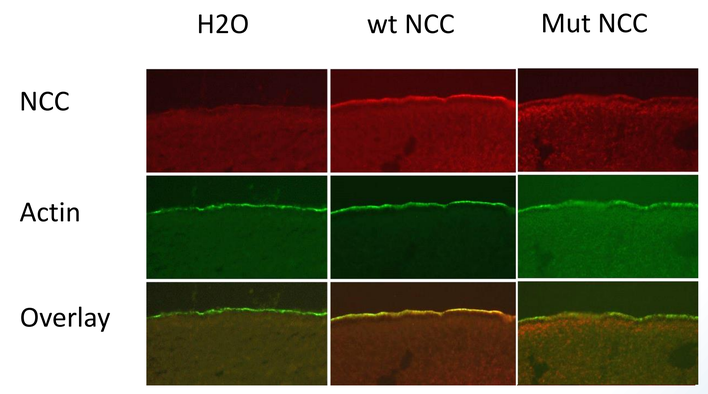


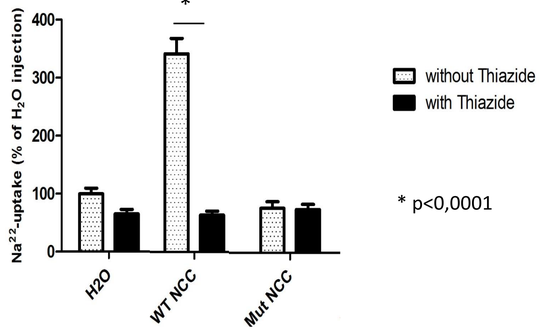


Western blots show more expressed glycosylation bands in wild type than in mutated NCC at 24 (p=0,003 NCC wt mean + SEM 0,233±0,046; NCC mut mean + SEM 0,0268±0,008) and 48 hours after transfection (p=0,0003 NCCwt 0,524±0,084; NCC mut 0,059±0,012), suggesting impaired maturation of the NCC mutated protein



Xenopus Laevis oocytes





* p<0,0001

Using antibodies against NCC, in oocytes expressing NCC wt we observed a staining for the protein at the surface of the oocytes. By contrast, in those injected with NCC mut RNA, antibody against NCC was detectable under the surface of the oocytes. A co-labeling with actin antibody was performed to highlight the cortex portion as housekeeping marker

In physiological conditions, controls group absorbed a low quantity of $^{22}Na^+$ set as 100 % and when challenged with thiazide treatment showed less, but not significant, uptake (without thiazide 100 vs. with thiazide 65.01 p>0.05). NCC wt oocytes could absorb $^{22}Na^+$ more than 3 folds compared to controls (342.3) When thiazides were added to NCC wt, the cotransporter activity was significantly inhibited resulting in a drop of uptake (without thiazide 342.3 vs. with thiazide 63.3 p<0.0001). By contrast, NCC mut could not uptake 22Na+ in both the two conditions with or without thiazides (without thiazide 77.3 vs. with thiazide 75 p>0.05).

Conclusions

We identified a novel GS causing point mutation that diminishes NCC function due to an impaired trafficking of the protein to the cell surface. The absence of any mature glyocosylation of mutated NCC suggests that the mutation impairs protein folding leading to a retention of NCC in the endoplasmic reticulum.

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