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Elucidating the Function of WNKs on Pseudohypoaldosteronism Type II Caused by KLHL3 BTB Domain Mutation

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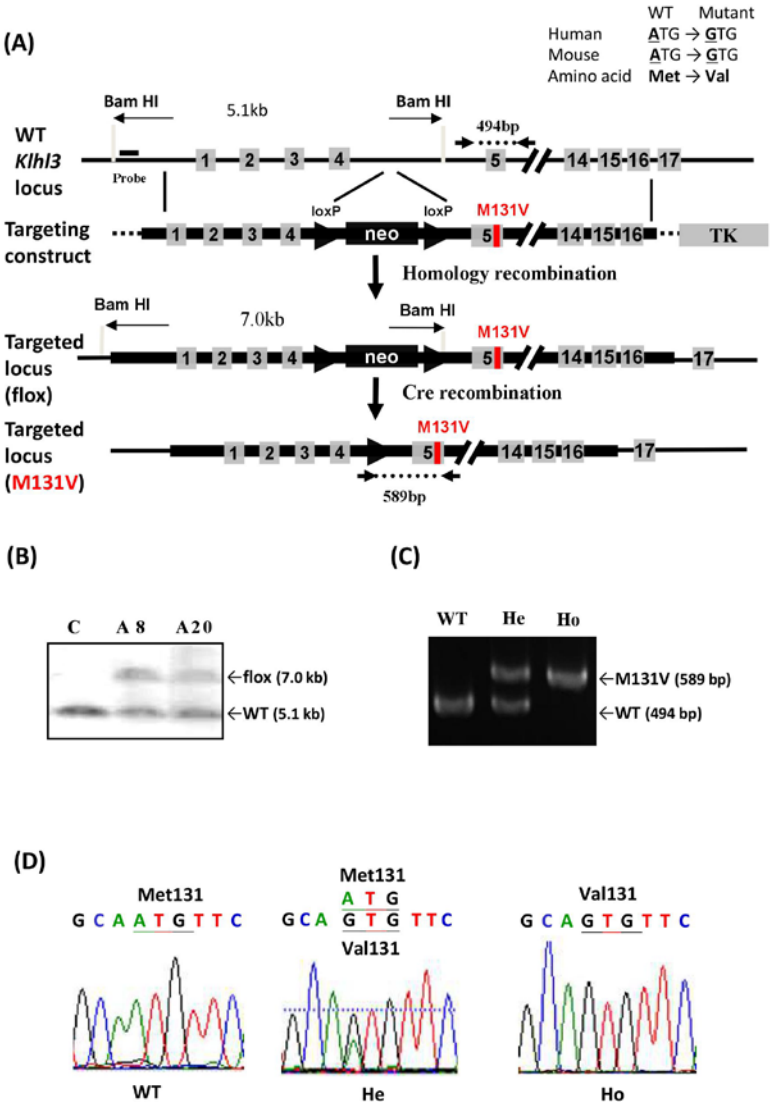
Objectives: Enhanced SPAK/OSR1-NCC cascade caused by mutations in Kelch-like 3 (KLHL3) or Cullin3 (Cul3) involved in WNK1/4 ubiquitination is known to cause pseudohypoaldosteronism type II (PHAII). It remains unclear that which WNK kinases is the major regulator in *KLHL3* mutation-causing PHAII.

Methods: We generated and analyzed *Klhl3* knock-in (KI) mice carrying a missense *M131V* mutation in the BTB domain (corresponding to human *KLHL3 M78V* mutation) and further crossed them with *Wnk4* $^{-/-}$ mice generated by targeting disruption from the promoter to exon 2 of *Wnk4*. The molecular mechanisms of PHAII regulated by *KLHL3* BTB domain mutation were further evaluated by western blot, immunogold labelling, qRT-PCR in microdissected renal tubules, and co-immunoprecipitation (Co-IP).

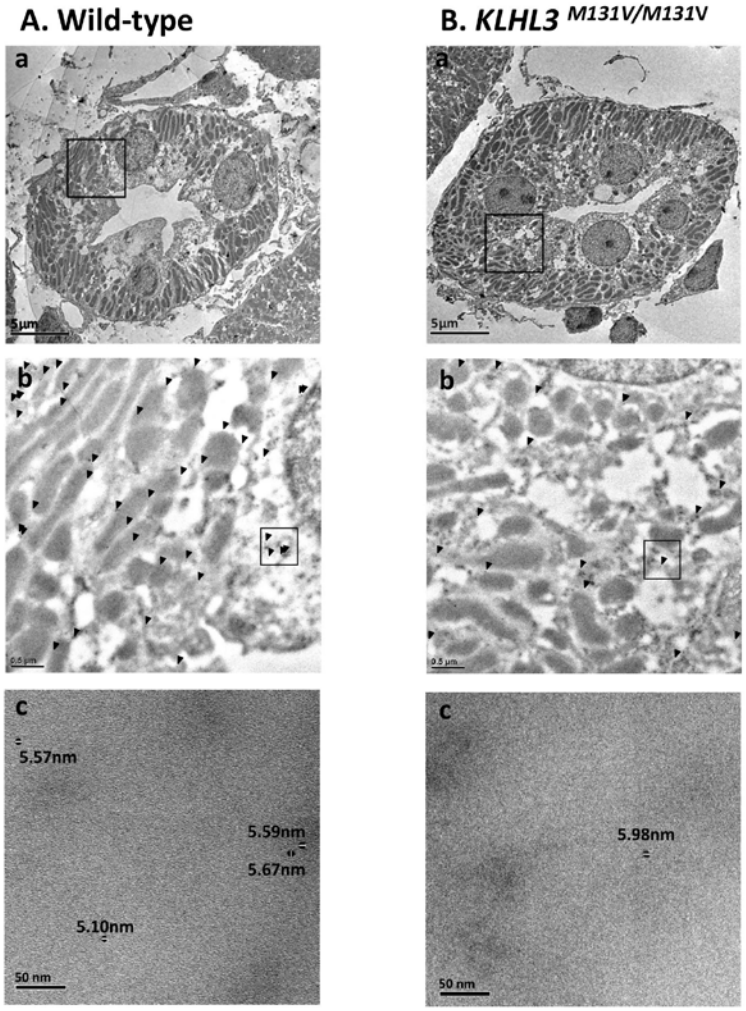
Results: Klhl3$^{M131V/+}$ KI mice exhibited typical feature of PHAII with hypertension with suppressed PRA and hyperkalemic metabolic acidosis. Their kidney tissues showed an unchanged KLHL3, decreased Cul3, and increased WNK1/4 expression along with an enhanced downstream SPAK/OSR1-N(K)CC phosphorylation. Their Cul3 protein expression in the cytosol of distal convoluted tubules cells was significantly attenuated on immunogold labelling electron microscopy. In microdissected renal tubules, *Klhl3*$^{M131V/+}$ KI mice expressed high levels of *Wnk4 mRNA* in the distal nephron. *In vitro* Co-IP showed the *KLHL3* BTB domain mutation retained intact interaction with WNKs but reduced binding to Cul3, thus leading to the increased abundance of total WNKs. *Klhl*$^{M131V/M131V}$. *Wnk4* $^{-/-}$ double transgenic mice still showed the overwhelming phenotypes of Gitelman syndrome (GS) with an increased expression of WNK1 but a decreased phosphorylation of SPAK and NCC.

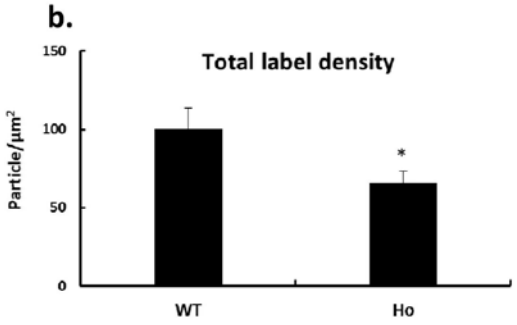
Conclusions: *Klhl3*$^{M131V/+}$ KI mice feature typical PHAII with a simultaneous increase of WNK1 and WNK4 through the impaired KLHL3 BTB domain binding to Cul3 and fail to correct the GS-like phenotypes of the *Wnk4* $^{-/-}$ mice. WNK4 is a vital WNKs in regulating SPAK/OSR1-N(K)CC signalling cascade under the circumstances of *KLHL3* BTB domain mutation.

Generation of Klhl3M131V/+ KI mice



Reduced expressions of Cul3 in DCT cells of Klhl3M131V/+ KI mice


A. Wild-type
B. KLHL3 M131V/M131V
a
b
c
5μm
5.57nm
5.59nm
5.67nm
5.10nm
50 nm
5.98nm


b.
Total label density
Particle/μm²
150
100
50
0
WT
Ho
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