An inducible transgenic mouse model for familial hypertension with hyperkalaemia (Gordon’s syndrome or pseudohypoaldosteronism type II)

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Abstract
Mutations in the novel serine/threonine WNK [With No lysine (∼K)] kinases WNK1 and WNK4 cause PHAII (pseudohypoaldosteronism type II or Gordon’s syndrome), a rare monogenic syndrome which causes hypertension and hyperkalaemia on a background of a normal glomerular filtration rate. Current animal models for PHAII recapitulate some aspects of the disease phenotype, but give no clues to how rapidly the phenotype emerges or whether it is reversible. To this end we have created an inducible PHAII transgenic animal model that expresses a human disease-causing WNK4 mutation, WNK4 Q565E, under the control of the Tet-On system. Several PHAII inducible transgenic mouse lines were created, each with differing TG (transgene) copy numbers and displaying varying degrees of TG expression (low, medium and high). Each of these transgenic lines demonstrated similar elevations of BP (blood pressure) and plasma potassium after 4 weeks of TG induction. Withdrawal of doxycycline switched off mutant TG expression and the disappearance of the PHAII phenotype. Western blotting of microdissected kidney nephron segments confirmed that expression of the thiazide-sensitive NCC (Na⁺–Cl⁻ co-transporter) was increased, as expected, in the distal convoluted tubule when transgenic mice were induced with doxycycline. The kidneys of these mice also do not show the morphological changes seen in the previous transgenic model expressing the same mutant form of WNK4. This inducible model shows, for the first time, that in vivo expression of a mutant WNK4 protein is sufficient to cause the rapid and reversible appearance of a PHAII disease phenotype in mice.

Key words: hyperkalaemia, kidney, monogenic syndrome, salt-dependent hypertension, solute carrier family (SLC) co-transporter, thiazide diuretics

INTRODUCTION
Hypertension affects one quarter of the world’s industrialized population and presents a significant risk factor for stroke, heart disease and renal failure. For most individuals, their major factor for developing hypertension is the inheritance of multiple genetic risk alleles whose individual effects on BP (blood pressure) are small and modulated by gene–gene and gene–environment interactions. However, there are rare familial forms of hypertension that are inherited through a single mutant pressor gene. PHAII [pseudohypoaldosteronism type II; OMIM Online Mendelian Inheritance in Man (145260)] or Gordon’s syndrome (PHAII) exemplifies one of these monogenic forms of familial hypertension and is caused by mutations that cause a gain-of-function in the thiazide-sensitive NCC (Na⁺–Cl⁻ co-transporter) in the distal nephron of the kidney. The mutations involve either novel serine/threonine kinases that regulate the trafficking and phosphorylation of NCC, WNK1 and WNK4 [where WNK is With No lysine (∼K)] [1,2] or two recently identified genes thought to be involved in the ubiquitination of NCC [3]. Subjects carrying these mutations have salt-dependent hypertension and hyperkalaemia that is characteristically reversed by small doses of thiazide-type diuretics that block the enhanced NCC transporter activity [4,5].

Previous use of transgenic and knock-in approaches have shown that expression of a mutant WNK4 protein in mice can mimic the human PHAII phenotype with mouse models showing elevated BP and hyperkalaemia [6,7]. However, this is not a universal experience, and transgenic mice expressing the D561A WNK4 mutant in the distal nephron do not have a PHAII phenotype [8]. A recent report has also highlighted that transgenic
overexpression of NCC in the mouse kidney does not cause a PHAII phenotype or even raise the BP [9]. There is also no data on how quickly a PHAII phenotype emerges when a mutant WNK4 protein is expressed in vivo and what happens to the phenotype when mutant protein expression ceases.

To address these issues we aimed to conditionally induce and reverse a PHAII phenotype by using a mouse carrying a TG (transgene) expressing the Q562E mutation in the WNK4 gene (referred to subsequently as WNK4 Q562E), the murine equivalent of the known human Q562E mutation of WNK4, using the Tet-On system [10–12]. The Tet-On system relies on a transactivator protein, rtTA, which is responsible for binding to a TRE (tetracycline response element) in the presence of the tetracycline-derivative, doxycycline. The rtTA is normally supplied as part of the Tet-On plasmid vector and delivered in trans, i.e. as a separate plasmid transfected into the target cell type bearing a pTRE2 plasmid. Alternatively, for in vivo work, the delivery is effected by crossing two animals, one carrying the pTRE2 response plasmid and the other the Tet-On for transactivation. However, in the model reported here the pTRE and rtTA were expressed in a single expression cassette driving the WNK4 Q562E cDNA expression in the presence of doxycycline. Several transgenic mouse lines were created with this construct and all develop a typical PHAII phenotype of hypertension and hyperkalaemia when exposed to doxycycline, which completely reverses when the doxycycline is withdrawn. This shows that expression of the mutant WNK4 Q562E gene is sufficient to cause the emergence of a PHAII phenotype in 4 weeks and provides a useful reversible mouse model for this familial hypertension syndrome.

**MATERIALS AND METHODS**

**RNA extraction and cDNA synthesis**

RNA was extracted from whole mouse kidney using TRIzol® (Invitrogen), according to manufacturer’s instructions, and only samples with a $A_{260}/A_{280}$ ratio of at least 1.8 on a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific) were processed. The cDNA was reverse-transcribed using Oligo (dT) primers (Invitrogen) and, SuperScript® III Reverse Transcriptase (Invitrogen) using 1 µg of RNA, according to manufacturer’s instructions.

**Creation of the tetracycline-inducible WNK4 Q562E cDNA construct**

RNA isolated from homogenized mouse kidney was transcribed to synthesize mouse WNK4 cDNA. A 4117 bp WNK4 cDNA was then amplified by PCR, using forward 5′-AT-TATACGCGGTAACCAGCGGTCGCTGCTGACCGCAGCA-ACCTCCAG-3′ and reverse 5′-ATTATTCTAGAATTTCGAGCCCTTTCTCCATTTTTATGGTGCTTGTA-3′ primers, 10 mΜ dNTP mix (Bioline), 2.5 units of Pfu Turbo® Hotstart DNA polymerase (Agilent Technologies), 10× reaction buffer and 50 ng of template cDNA. An MluI restriction enzyme site was incorporated into the 5′ end of the forward primer and XhoI and XbaI restriction enzyme sites into the 5′end of the reverse primer. Thermal cycling was: one cycle at 95°C for 5 min, 35 cycles of 95°C for 30 s, 68°C for 1 min and 72°C for 5 min. The product was purified by a QiAquick® PCR Purification Kit (Qiagen) and ligated into the MCS (multiple cloning site) of a compatible restriction digested pTRE2 vector (Clontech). This plasmid was then subjected to site-directed mutagenesis PCR using a QuickChange® Site-Directed Mutagenesis Kit (Qiagen). Forward (5′-GAGCCAGGGCTGACGAGCATCAATCCCTCC-3′) and reverse (5′-GGAAGGTATGATGCTGCACCTTCGTGCTGTCGTC-3′) primers were used to introduce the C→G substitution creating the Q562E mutation. The final WNK4 Q562E cDNA was downstream of the enhancerless minimal CMV (cytomegalovirus) promoter (P mini CMV) fused to an upstream TRE. The entire miniCMV TRE WNK4 Q562E cDNA was excised using XhoI, and cloned in-frame and downstream of the EGFP (enhanced green fluorescent protein) sequence STOP codon and into the XhoI restriction site within the MCS of the 4.7 kb pEGFP-C1 vector (Clontech). Using the Tet-On plasmid (Clontech) and forward (5′-GGTTGACTTGATGTAGCTGACTTTAATA-GTAACTATT-3′) and reverse (5′-AAGCTTGTGGCA-GGTGATACCTCCCGTCGCCAGGGACAT-3′) primers, a 2169 bp fragment, consisting of an upstream enhancerless CMV promoter (P mini CMV), fused to an rtTA sequence with a downstream poly(A) tail was produced by PCR. The PCR product was checked on a 1% (w/v) agarose gel and purified using a Quick Spin™ Column (Roche). This rtTA-fragment was then cloned into a compatible SalI restriction site upstream of the TRE WNK4 Q562E cDNA, within the modified pEGFP-C1 plasmid. Since EGFP expression has been shown to cause cardiomyopathy in mice [14], the CMV sequences driving EGFP expression were excised as a 3460 bp fragment using AgeI and DraIII enzymes (New England Biolabs). This final linearized WNK4Q562E TG construct was microinjected into oocytes (Figure 1).

**Generation of transgenic mice**

All animal procedures had local ethical committee approval and were carried out in accordance with UK Home Office regulations. C57BL/6J and CBA/J mice (both from Charles River Laboratories) were crossed to generate B6CBA/F1 hybrid mice to allow the harvesting of single-cell B6CBA/F2 fertilized eggs for manipulation in vitro. Transgenic mice were generated by pronuclear microinjection of the linearized WNK4 Q562E cDNA TG construct into B6CBA/F2 single-cell mouse embryos according a standard method [15,16]. Following pronuclei fusion, zygotes were allowed to divide to a two-cell stage in vitro and then implanted into pseudopregnant females. The resulting pups were genotyped by PCR to identify transgenic founders (see below).

**Genotyping of mice**

Transgenic founders were identified using primers to distinguish between WT (wild-type) genomic WNK4 sequence and the WNK4 TG. Forward (5′-GAAGTGAGGCACACTAGACAC-3′) and reverse (5′-CGTACTCAGAGTGTCCTCC-3′) primers were designed to hybridize to WNK4 exons 17 and 18 respectively. This amplified TG cDNA and WNK4 genomic sequences as separate PCR products: a 221 bp and a
larger 371 bp product due to the 150 bp intron 17 (Figure 2A). The Q562E mutation in transgenic mice was confirmed by PCR and restriction digest of the products since the Q562E mutation creates a Hin4I restriction site. Exon 7 was PCR amplified using forward (5'-GAGAACGGGTGCTGCTATC-3') and reverse (5'-TTGATGAGTAGCTGGCATGG-3') primers and the 204 bp product digested with Hin4I and run on a 2% (w/v) agarose gel. WT mice contained only the 204 bp product from genomic DNA, whereas transgenic mice showed additional bands (153, 32 and 19 bp) reflecting Hin4I digestion of the 204 bp PCR product derived from the TG WNK4 Q562E cDNA (Figure 2B).

**TG copy number determination**

The number of WNK4 Q562E cDNA TG copies in each line was determined using a real-time TaqMan® qPCR assay. Primer and probe sets were used to detect WNK4 cDNA TG and the KCNJ1 control gene, which encodes the inwardly-rectifying potassium channel protein ROMK. Genomic DNA extracted from WT and transgenic founder mice was quantified using NanoDrop® to produce a standard of 100 ng/μl and diluted to give DNA standards from 100 to 0.1 pg/μl. The 1 μl triplicate samples of each standard, together with appropriate positive, WNK4 Q562E construct cDNA, and negative non-template controls were subjected to real-time qPCR (quantitative PCR) using an ABI Prism 7700 machine (Applied Biosystems) and appropriate primer/probe combinations. For the KCNJ1 control gene the expression primer/probe Mm00444727_s1 (Locus NM_019659; Applied Biosystems) was used to amplify a 126 bp fragment of exon 2. To detect the WNK4 Q562E cDNA TG, probe Mm00841400_m1 (Locus NM_175638; Applied Biosystems) was used to selectively amplify an 83 bp fragment spanning exons 4–5 of the cDNA. For KCNJ1, the mean fluorescence threshold, \( C_{t} \), was plotted semi-log against control genomic DNA in the dilution series to give a standard curve from 100 ng–0.1 pg. This was repeated for the WNK4 Q562E cDNA TG and the shift in the two curves, \( \Delta C_{t} \), was used to estimate the
copy number of WNK4 Q562E TGs integrated into each founder genome as $2 \times (2^{\Delta C_t})$.

**Breeding of transgenic lines**

WNK4 Q562E transgenic founders were bred with non-transgenic WT B6CBA mice to generate three individual lines. Hemizygotes for each line were identified by PCR (see below) and crossed to generate homozygotes. Transgenic homozygotes (TG/TG) identified by PCR (see below) were then crossed to establish homzygous lines of animals for phenotyping studies. The zygosity of hemi- against homo-zygotes was determined by a real-time quantitative TaqMan® assay using the above mouse WNK 4 probe and primers (Mm00841400_m1; Applied Biosystems). TG/TG mice have twice the amount of TG cDNA as their hemizygote counterparts, which was detected by the one $C_t$ cycle difference between them. TaqMan® assays were run in triplicate and genomic DNA from non-transgenic mice were used as negative controls. Transgenic homozygotes in each of the three lines were interbred to generate the final lines: low TG, mid TG and high TG copy number mice. Homozygotes from each line were used for phenotyping.

**Gene expression assays**

Kidney RNA was extracted from mice and reverse transcribed as above (on or off doxycycline) The resulting cDNA was probed using primer/probe sets to quantify expression of WNK4 (Mm00841400_m1), SLC12A3 (Mm00490213_m1) and KCNJ1 (Mm01173990_m1) (all from Applied Biosystems). Expression levels were normalized to the expression of the $\beta$-actin housekeeping gene, using the primer/probe set Mm 00607939_s1(Applied Biosystems).

**Animal and treatments**

Age-matched male and female WNK4 Q562E transgenic and control B6CBA mice (WT/WT) were placed on either normal diet containing doxycycline at 200 mg/kg of body weight or stored at $20^\circ C$ overnight to visualize either: $\beta$-actin (housekeeping protein), NCC protein or pNCC (phosphorylated NCC) at Thr58. The antibodies used were polyclonal goat anti-(mouse $\beta$-actin) antibody (Abcam) at 1 $\mu$g/ml, polyclonal rabbit anti-(rat NCC) antibody (Chemicon) at a dilution of 1:2000, and polyclonal sheep anti-(human pNCC Thr58)antibody (kindly provided by Professor Dario Alessi, MRC Phosphorylation Unit, University of Dundee, Dundee, Scotland, U.K.) at a concentration of 2 $\mu$g/ml. Blots were visualized using ECL (enhanced chemiluminescence) reagent (Amersham Biosciences) according to the manufacturer’s instructions.

**RESULTS**

**Generation of WNK4 Q562E transgenic mice**

A total of 13 mice were successfully born after pronuclear microinjection of the cloned and linearized WNK4 Q562E TG construct into recipient mouse oocytes. The genotyping PCR (Figure 2) showed that six of these 13 founder (F0) mice had the WNK4 Q562E TG detectable in their genome. The TG copy number in these founders was measured by real-time TaqMan® PCR with KCNJ1 as the reference gene. The $C_t$ for amplification of this gene in serial dilutions of a mouse genomic DNA standard was determined. Similar curves were generated using a WNK4 TaqMan® probe set against serial genomic DNA dilutions from each founder mouse and are plotted on the same KCNJ1 curve.
From the difference in cycle number, \( \Delta C_t \), between the WNK4 and KCNJ1 curves copy number was estimated for each founder (Table 1). From these results, three founders were taken forward to produce lines containing low- (founder 2), medium- (founder 5) or high- (high founder 6) TG copy number. During the breeding of these lines the zygosity status of the offspring was established by real-time TaqMan\textsuperscript{®} PCR assay for the WNK4 Q562E TG. Homozygote mice from these three lines were then used in further phenotyping.

**TG expression levels**

To confirm TG activation whole kidney levels of WNK4 RNA transcript were measured by qPCR in mice from each of the 3 lines before and after 1 month on a doxycycline-containing diet (Figure 3). The levels of WNK4 RNA in non-transgenic mice were not affected by the doxycycline diet itself, but it caused a 16-, 87- and 151-fold increase in WNK4 RNA levels in the low-, middle- and high-TG copy number lines respectively. These levels reversed after a further 1 month on the doxycycline-free control diet to pre-stimulation levels. The baseline levels of WNK4 RNA were not different between the non-transgenic control mice (WT/WT) and the low-TG copy number line, but were \( \sim \)2-fold higher in the mid- and high-expressing lines, suggesting some leak from the TG due to the high copy numbers (Figure 3). The levels of transcript for NCC (Sc12a3) in the kidney were not affected by exposure to doxycycline or different in any of the lines compared with non-transgenic control mice (Supplementary Figure S1 at http://www.clinsci.org/cs/124/cs1240701add.htm).

**FISH**

To confirm chromosomal integration of the TG, FISH was performed on the low TG copy number mouse. This confirmed integration on the homologue of chromosome 4 (Figure 4). The fluorescent probe did not hybridize to the non-transgenic mouse metaphase chromosomes, confirming the specificity of the probe for the WNK4 Q562E TG.

**BP and plasma K\(^{+}\) measurement**

An elevated plasma K\(^{+}\) concentration is a striking feature of the PHAII phenotype that distinguishes it from other monogenic hypertension syndromes. In parallel with the WNK4 Q562E TG activation, all three transgenic lines showed significantly elevated plasma K\(^{+}\) levels after 1 month on the doxycycline-containing diet compared with mice fed on the control diet (Figure 5A). Silencing of the TG by a further month on the doxycycline-free diet led to normalization of the elevated plasma K\(^{+}\) levels in all three lines (Figure 5A). In a parallel fashion the low- and mid-TG copy number lines had a significant elevation of their mean arterial BP of \( \sim \)20 mmHg after being dosed with doxycycline for 1 month (Figure 5B). However, the increase did not reach statistical significance in the high-expressing line. Dosing for a further month on a doxycycline-free diet returned the BP to the levels observed in the non-transgenic controls (Figure 5B).

**Western blot analysis of NCC expression from microdissected kidney tubules**

Expression of total NCC and pNCC protein was studied in microdissected tubules from the low-TG copy number line only. Protein staining for NCC and pNCC was only detected in the microdissected tubules from the DCT (distal convoluted tubule) and both were increased \( \sim \)1.5–2-fold in low-TG copy number mice after 1 month on the doxycycline-containing diet (Figure 6).
DISCUSSION

The results of the present study show that it is possible to create an inducible model of Gordon’s syndrome (PHAII) by expressing a mutant WNK4 protein in vivo under a Tet-On system. The previous work has shown that mice constitutively expressing WNK4 Q562E or another mutant as a knock-in (D561A +/−) have hypertension and hyperkalaemia [6]. TG expression of the same D561A mutation in another mouse model, however, did not produce a PHAII phenotype [8], and it is not clear why these mouse models behave differently. Differences in the genetic background of the mice used may be important. Another factor that has emerged in some WNK1 mouse models of Gordon's syndrome (PHAII) is compensatory changes to other parts of the nephron to explain the lack of the expected phenotype [21]. The phenotypes of adults with the WNK4 Q562E mutation can be distinct from children even within the same pedigree [22].

In our model, there was high-level expression of the TG following doxycycline activation particularly within the mouse with the highest high TG copy number. However, differences in TG expression did not translate into any obvious differences in the degree to which the BP or plasma K+ levels increased on doxycycline activation of the TG. The BP in the high copy number line did not increase significantly on doxycycline, although the plasma K+ levels did. The significant fall in BP off doxycycline suggests this lack of a significant BP increase may actually have been a false negative result. The lack of an obvious TG dose-effect is perhaps not surprising as Gordon’s syndrome (PHAII) is an autosomal dominant condition and the phenotype emerges from more or less balanced levels of WT and mutant WNK4 transcripts in vivo. However, there was evidence for low-level constitutive TG activation in the mice with middle and high copy numbers (Figure 3). This leaky behaviour is well described for the Tet-On system, so future phenotypic work should probably focus on the low copy number line to reduce the impact of this leakiness. Nevertheless, the high levels of TG activation seen in our mice did not directly affect the level of Slc12a3 mRNA expression or lead to ectopic NCC expression in immunostained kidney sections (see Supplementary Figures at http://www.clinsci.org/cs/124/cs1240701add.htm). In fact, the level of NCC expression within microdissected DCT tubules in the mice with a low copy number was increased as expected and compares closely to the 2-fold increase reported in whole kidney blots from the D561A knock-in mouse model [7].
In a previous constitutive transgenic mouse expressing the Q562E mutation, the adults showed obvious structural changes to their distal nephron with marked enlargement of the DCT lumens in kidney sections [6]. Even if the density of NCC at the apical membrane of these tubules were unchanged their increased surface area could substantially increase total Na$^+$–Cl$^-$ co-transport through NCC. There are no reports of the histology of the kidney in human subjects with Gordon’s syndrome (PHAII) to say whether similar morphological changes in the DCT occur in subjects carrying WNK4 mutations. However, similar changes were not seen in our transgenic mice at baseline or after after 4 weeks of TG induction. In fact, histological sections of the kidney from our mice after a month of doxycycline induction of the TG were indistinguishable from WT mice (see Supplementary Figure S2 at http://www.clinsci.org/cs/124/cs1240701add.htm). This makes it unlikely that the structural changes previously reported in mice constitutively expressing mutant WNK4 are important for a Gordon’s syndrome (PHAII) phenotype.

There is now a large body of data showing that WNK4 does not work by simply phosphorylating NCC [23]. It actually sits within a signalling cascade and phosphorylates a downstream kinase called SPAK. Activated SPAK then phosphorylates key serine/threonine residues in the N-terminus of NCC causing its activation in vitro, and phosphorylation in vivo may be restricted to NCC inserted into the apical membrane of DCT cells. It also independently reduces forward trafficking of NCC from the Golgi by accelerating its lysosomal degradation [24]. This complexity to the molecular actions of WNK4 probably explains why a transgenic mouse overexpressing NCC can have a nor-

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We have not investigated whether shorter exposure times to doxycycline and hence TG activation would be as effective as the 4-week schedule chosen. However, there are useful clinical clues on this issue assuming TG activation and silencing parallels the time course of responses of subjects with Gordon’s syndrome (PHAII) to a thiazide diuretic. The activated NCC transporter function caused by mutant WNK4 in these subjects makes affected subjects sensitive to even low doses of thiazide diuretics. In fact, the PHAII phenotype, especially the hyperkalaemia, resolves in 1–2 weeks of commencing treatment with a thiazide [5,26], and if the thiazide is discontinued re-emerges within 2 weeks, presumably reflecting ongoing expression of mutant WNK4 protein [5]. So, our mouse model based on 4 weeks of TG activation producing a PHAII phenotype and its recovery within 4 weeks of silencing the TG recapitulates well the clinical behaviour of subjects with Gordon’s syndrome (PHAII) exposed to a thiazide diuretic. Of course, transient expression of mutant WNK4 does have potential limitations. For example, mutant WNK4 expression does have effects outside of the kidney, such as a loss of bone mineral density that could reflect chronic or even lifelong hypercalciauria [5]. This has not been reported so far in any mouse model of Gordon’s syndrome (PHAII), but clearly a model with only transient TG activation is not suited to explore this extended PHAII phenotype in the mouse.

In summary then, we have produced a robust inducible mouse model of the rare monogenic disorder, Gordon’s syndrome (PHAII). The model shows that expression of mutant WNK4 is able to recapitulate the features of hypertension and hyperkalaemia after just 4 weeks of TG activation and that it is completely lost after a further 4 weeks of TG silencing. It should be useful in further exploration of the molecular physiology of the distal nephron in Gordon’s syndrome (PHAII).

**CLINICAL PERSPECTIVES**

- The monogenic syndromes that cause familial hypertension are rare but unravelling their molecular basis has provided fundamental insights into how the distal nephron works. Gordon’s syndrome (PHAII) is one of these monogenic syndromes. In the present paper, we report a new transgenic mouse model for the syndrome that is inducible by doxycycline.
- Inducing the TG causes expression of a mutant form of WNK4 leading to activation of the NCC transporter that is the usual target for thiazide diuretics in the kidney. This drives an increase in BP and plasma K$^+$ levels after 4 weeks that mimic closely those seen clinically in Gordon’s syndrome (PHAII). It is reversed on withdrawal of the doxycycline for the same period and not accompanied by the morphological changes previously observed in a constitutively active transgenic model.
- This model should be useful in gaining a better understanding of the molecular basis for Gordon’s syndrome (PHAII) and its impact on the distal nephron.

**AUTHOR CONTRIBUTION**

Jabed Chowdhury performed most of the experiments, Che-Hsiung Liu designed and made the transgene, Annie Zuber performed the microdissection, and Kevin O'Shaughnessy conceived the study and wrote the paper.

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SUPPLEMENTARY ONLINE DATA

An inducible transgenic mouse model for familial hypertension with hyperkalaemia (Gordon’s syndrome or pseudohypoaldosteronism type II)

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<th>Figure S1 Total kidney NCC (SLC12A3) transcript levels in the three WNK4 Q562E transgenic mouse lines</th>
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<td>Before (-dox), after (+dox) or following doxycycline removal (off dox). Levels are normalized to levels in WT control mice kidney (-dox). Bars are mean transcript levels ± S.E.M for n = 8–10 mice.</td>
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Correspondence: Dr Kevin M. O’Shaughnessy (email kmo22@medschl.cam.ac.uk).
Figure S2 Representative low-power (A) or high-power (B and C) fields of whole-mouse kidney sections immunostained for (A and B) NCC and pNCC (at Thr58) (C) from control mice (WT/WT) or transgenic mice (Tg/Tg) either off or after 4 weeks of a doxycycline-containing diet.

The low-TG line was used for these studies.