Supplemental Data

A novel mouse model of X-linked nephrogenic diabetes insipidus: Phenotypic analysis and therapeutic implications

Jian Hua Li, Chung-Lin Chou, Bo Li, Oksana Gavrilova, Christoph Eisner, Jürgen Schnermann, Stasia A. Anderson, Chu-Xia Deng, Mark A. Knepper, and Jürgen Wess

Supplemental Methods

Metabolic cage studies. Animals were maintained in mouse metabolic cages (Hatteras Instruments, Cary, NC) under controlled temperature and light conditions (12 hr light and dark cycles). Mice received a fixed daily ration of 6.5 g of gelled diet per 20 g of body weight per day. The gelled diet was composed of 4 g of Basal Diet 5755 (Test Diet, Richmond, IN), 2.5 ml of deionized water, and 65 mg agar. Preweighted drinking water was provided *ad libitum* during the course of the study. Mice were acclimated in the metabolic cages for 1-2 days. Urine was collected under mineral oil in preweighted collection vials for successive 24 hr periods.

Analysis of GPCR expression in mouse IMCD cells via TaqMan real-time qRT-PCR. Total RNA prepared from mouse IMCD tubule suspensions was reverse transcribed as described under Experimental Procedures. Tissues from ten 10-week old C57BL/6 WT mice were collected and pooled for each individual experiment. cDNA derived from 640 ng of RNA was mixed with an equal volume of TaqMan gene expression 2 x master mix (Applied Biosystems, Foster City, CA). 100 µl-aliquots of this mixture (corresponding to 80 ng of RNA) were added to each of the 8 fill ports of a 384-well plate of a mouse GPCR array panel (Applied Biosystems). Real-time qRT-PCR measurements were carried out using the 7900HT Fast Real-Time PCR System (Applied Biosystems). The following PCR conditions were used: 97 ºC for 30 sec, followed by 40 cycles at 59.7 ºC.

Delivery of ONO-AE1-329 (ONO) via osmotic minipumps. Because of poor water solubility, ONO was dissolved in a saline/DMSO mixture (50%/50%) at a concentration of 2.3 μg/μl. The ONO solution was infused s.c. via osmotic minipumps (Alzet model 2001, reservoir volume 0.2 ml). Each mouse received 55 μg of ONO per day corresponding to 24 μl of ONO solution. Minipumps were implanted subcutaneously between the scapulae of adult mice five days after the last TMX injection. Infusions were carried out for up to 3 weeks. Because of the low solubility of the ONO compound, minipumps had to be replaced weekly.

Blood pressure and pulse measurements. We used a non-invasive procedure to monitor blood pressure (BP) in conscious mice. This method involved the use of a tail cuff equipped with a pressure transducer using a totally automated, computer-controlled BP analysis system (model MC4000; Hatteras Instruments, Cary, NC). This system provides greater accuracy and precision than previous instruments designed for measuring BP via the use of tail cuffs. Conscious mice were restrained in a chamber designed to provide a comforting environment to minimize stress and render measurements more reliable. Systolic, diastolic, and mean arterial BP as well as pulse frequency were recorded. Mice were acclimated to the procedure for 2-3 days. BP measurements were then taken during the following 2-3 days. During BP measurements, mice were awake and restrained in a dark chamber on a warmed platform, with their tails sticking out. The tail was passed through the tail cuff and the LED detector and was taped down.

Supplemental Table 1

Effect of TMX-induced deletion of the V2R gene on urine chemistry

	V2R fl/y Esr1-Cre mice	
	Before TMX	After TMX
Urea (mg/dl)	3,771 ± 96	237 ± 35***
Creatinine (mg/dl)	26.0 ± 1.0	6.6 ± 0.4***
Na^+ (mEq/l)	156 ± 6	10.4 ± 1.7***
K^+ (mEq/l)	139 ± 5	11.5 ± 2.2***

V2R fl/y Esr1-Cre mice were placed into metabolic cages and 24 hr urines were collected. Urine chemistry measurements were carried out immediately before or after TMX administration for 6 days (0.5 mg i. p./mouse/day). Data are given as means ± SEM (n = 5-7; ***$P < 0.001$).

Supplemental Table 2

List of GPCRs (or potential GPCRs) expressed in mouse IMCD tubule cells

IMCD tubule suspensions were isolated from 10 adult C57BL/6 WT mice (10-week old males) and GPCR mRNA expression levels were quantitated by TaqMan real-time qRT-PCR analysis. For experimental details, see Supplemental Methods. Only genes with C_t values <31 are listed. Data are given as means ± SEM of two independent experiments.

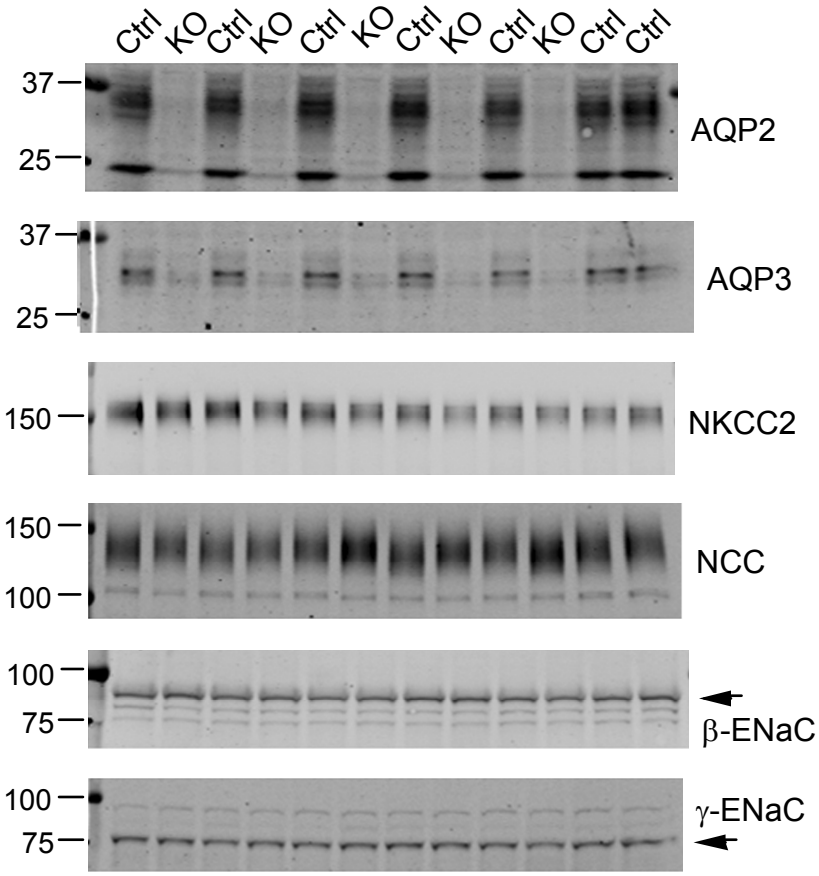
Gene symbol	Encoded protein	C_t
	Internal controls	
ACTB	β-actin	21.7 ± 0.1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	22.7 ± 0.2
PGK1	phosphoglycerate kinase 1	24.9 ± 0.1
	G protein-coupled receptors (7 TM proteins)	
Avpr2	V2 vasopressin receptor	24.1 ± 0.2
Gpr56	G protein-coupled receptor 56 (orphan)	25.4 ± 0.3
Gpr116	G protein-coupled receptor 116 (orphan)	25.8 ± 0.0
Ptger1	Prostaglandin E2 receptor, EP1 subtype	25.9 ± 0.1
Gprc5c	G protein-coupled receptor 5c (orphan)	26.0 ± 0.2
Gprc5b	G protein-coupled receptor 5b (orphan)	26.1 ± 0.2
Edg1	Sphingosine-1-phosphate receptor 1	26.8 ± 0.0
F2rl1	Proteinase-activated receptor 2 precursor (PAR2)	26.9 ± 0.1
Gpr108	G protein-coupled receptor 108 (orphan)	27.2 ± 0.1
Fzd6	Frizzled 6	27.5 ± 0.2
Eltd1	Latrophilin 7 TM domain-containing protein 1	27.7 ± 0.1
Gprc5a	G protein-coupled receptor 5a (orphan)	27.7 ± 0.1
Lgr4	Leucine-rich repeat-containing G-protein coupled receptor 4 (orphan)	27.7 ± 0.1
Gpr146	G protein-coupled receptor 146 (orphan)	27.8 ± 0.1
P2ry5	P2Y purinoceptor 5	27.9 ± 0.1
Fzd4	Frizzled 4	27.9 ± 0.2
Gpr137	G protein-coupled receptor 137 (orphan)	28.0 ± 0.1
P2ry14	P2Y purinoceptor 14	28.3 ± 0.2
Gpr4	G protein-coupled receptor 4 (orphan)	28.7 ± 0.2
Ednrb	Endothelin receptor type B	28.7 ± 0.2
Celsr2	Flamingo 1 (orphan)	28.9 ± 0.1
Frzb	Frizzled-related protein	28.9 ± 0.1

Tm7sf3	TM 7 superfamily member 3	28.9 ± 0.1
Gpr137b	G protein-coupled receptor 137B (orphan)	29.1 ± 0.1
Gpr125	G protein-coupled receptor 125 (orphan)	29.2 ± 0.2
Ptger3	Prostaglandin E2 receptor, EP3 subtype	29.5 ± 0.2
Avpr1a	V1a vasopressin receptor	29.5 ± 0.2
Gpr39	G-protein coupled receptor 39	29.7 ± 0.1
Gpr97	Probable G-protein coupled receptor 97 (orphan)	29.8 ± 0.2
Crcp	Calcitonin gene-related peptide-receptor component protein	29.8 ± 0.1
Adra2a	α-2a adrenergic receptor	29.8 ± 0.1
Celsr1	Protocadherin flamingo 2 (orphan)	29.9 ± 0.0
Smo	Smoothened homolog precursor	29.9 ± 0.1
F2r	Thrombin receptor	29.9 ± 0.2
Fzd5	Frizzled 5	30.0 ± 0.0
P2ry2	P2U purinoceptor 1	30.0 ± 0.1
Edg2	Lysophosphatidic acid receptor 1	30.0 ± 0.2
Calcrl	Calcitonin gene-related peptide type 1 receptor precursor	30.5 ± 0.3
Gpr109a	Nicotinic acid receptor	30.6 ± 0.2
Gpr175	G protein-coupled receptor 175 (orphan)	30.6 ± 0.2
Fzd1	Frizzled 1	30.6 ± 0.2
Admr	Adrenomedullin receptor	30.7 ± 0.2
Htr1b	5-Hydroxytryptamine (serotonin) 1B receptor	30.8 ± 0.1
Ccrl2	Chemokine (C-C motif) receptor-like 2 receptor (orphan)	30.9 ± 0.1
Edg4	LPA2 receptor	30.9 ± 0.0
Ptger4	Prostaglandin E2 receptor, EP4 subtype	30.9 ± 0.0
Lphn2	Latrophilin 2 (class B receptor, orphan)	30.9 ± 0.0
Gpr160	G protein-coupled receptor 160	30.9 ± 0.0

Supplemental Table 3

PCR primer pairs used for mouse genotyping studies

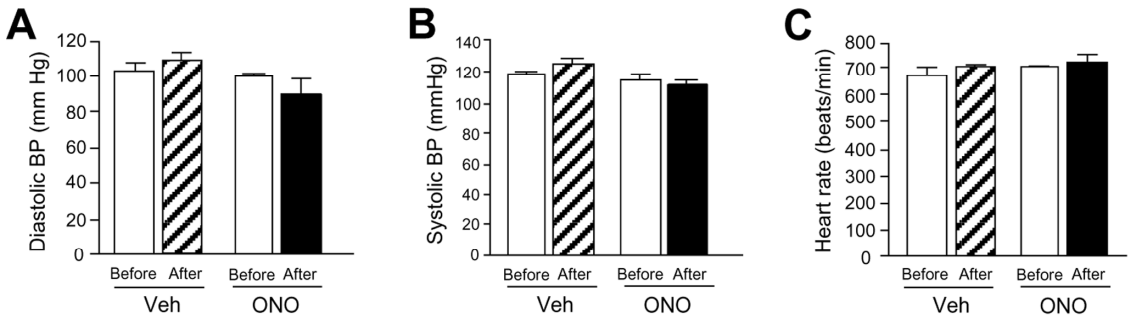
Primer	Sequence
Cre1	5'-CCTGGAAAATGCTTCTGTCCG (forward)
Cre2	5'-CAGGGTGTTATAAGCAATCCC (reverse)
p1	5’-TGATGAAGCTGAGCAGGCCCT (forward)
p2	5’-CTTGGTTCCCAACAGAGGGGT (reverse)
p3	5'-GAAGCTCCTCTGGAAAGTGGGT (forward)
p4	5'-TCCTATGAAGAAGAGAGACCAG (reverse)



Supplemental Figure 1

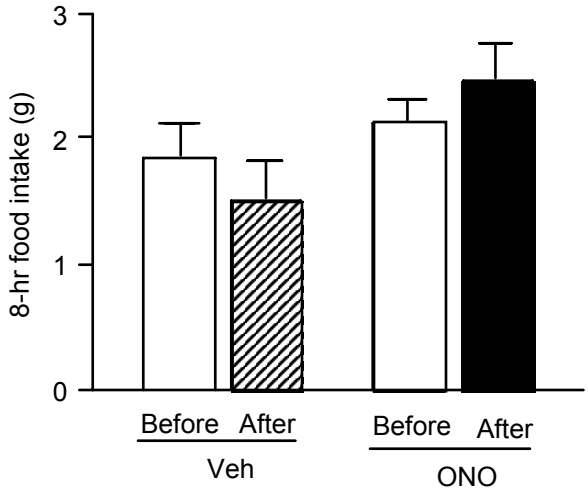
Immunoblots assessing protein abundance of the renal aquaporin water channels AQP2 and AQP3 and various renal sodium transporters in V2R KO and control mice

V2R KO mice (KO; TMX-treated *V2R fl/y Esr1-Cre* mice) and control littermates (Ctrl; TMX-treated *V2R fl/y* mice) were sacrificed 6 months after TMX treatment. Homogenates prepared from whole left kidneys were used for Western blotting studies. While AQP2 and AQP3 expression was drastically reduced in V2R KO mice, protein levels of NKCC2, NCC, β-ENaC, and γ-ENaC were not significantly affected by disruption of the V2R gene (V2R KO, n = 5; control, n = 7).



Supplemental Figure 2

ONO-AE1-329 (ONO) treatment has no effect on blood pressure and heart rate in V2R KO mice (**A, B**) Diastolic and systolic blood pressure (BP). (**C**) Heart rate. Heart rate (pulse frequency) was determined from variations in diastolic and systolic pressure. Measurements were carried out with V2R KO mice that had received either vehicle or ONO for 5-6 days. ONO (55 µg daily per mouse) or vehicle were infused s.c. via osmotic minipumps. Data are given as means ± SEM (n = 5 or 6 per group).



Supplemental Figure 3

ONO-AE1-329 (ONO) administration has no effect on food intake in V2R KO mice ONO **(**55 µg daily per mouse) or vehicle were administered to V2R KO mice via s.c. infusion for 9 days using osmotic minipumps. Food intake was measured for 8 hr in metabolic cages, either immediately before or 9 days after the start of the ONO or vehicle infusion. Data are given as means ± SEM (n = 4-6).