4. Pei Y, Watnick T, He N, et al. Somatic PKD2 mutations in individual kidney and liver cysts support a 'two-hit' model of cystogenesis in type 2 autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 1999;10:1524–1529.
5. Lantinga-van Leeuwen IS, Dauwerse JG, Baelde HJ, et al. Lowering of Pkd1 expression is sufficient to cause polycystic kidney disease. *Hum Mol Genet*. 2004;13:3069–3077.
6. Hopp K, Ward CJ, Hommerding CJ, et al. Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity. *J Clin Invest*. 2012;122:4257–4273.
7. Rossetti S, Kubly VJ, Consugar MB, et al. Incompletely penetrant PKD1 alleles suggest a role for gene dosage in cyst initiation in polycystic kidney disease. *Kidney Int*. 2009;75:848–855.
8. Bergmann C, von Bothmer J, Brüchle NO, et al. Mutations in multiple PKD genes may explain early and severe polycystic kidney disease. *J Am Soc Nephrol*. 2011;22:2047–2056.
9. Gilbert RD, Sukhtankar P, Lachlan K, et al. Bilineal inheritance of PKD1 abnormalities mimicking autosomal recessive polycystic disease. *Pediatr Nephrol*. 2013;28:2217–2220.

Treatment of Nephrogenic Diabetes Insipidus Patients With cGMP-Stimulating Drugs Does Not Mitigate Polyuria or Increase Urinary Concentrating Ability

Gitte R. Hinrichs[1,2,4], Line A. Mortensen[2,4], Claus Bistrup[2,3], Hans H. Dieperink[2,3] and Boye L. Jensen[1]

[1]Department of Molecular Medicine, University of Southern Denmark, Odense, Denmark; [2]Department of Nephrology, Odense University Hospital, Odense, Denmark; and [3]Department of Clinical Research, University of Southern Denmark, Odense, Denmark

Correspondence: Line Aas Mortensen, Department of Nephrology, Odense University Hospital, J.B. Winsløws Vej 4, 5000 Odense C, Denmark. E-mail: Line.Mortensen@rsyd.dk

[4]These authors contributed equally to this work.

Received 31 March 2020; revised 28 April 2020; accepted 4 May 2020; published online 23 May 2020

Kidney Int Rep (2020) **5,** 1319–1325; https://doi.org/10.1016/j.ekir.2020.05.015

Nephrogenic diabetes insipidus (NDI) is usually caused by a lack of responsiveness of the collecting ducts to the arginine vasopressin (AVP). It is characterized by excessive urinary output caused by impaired urinary concentration ability. In severe cases, adult patients excrete more than 10 L per day, making them at constant risk for severe dehydration. Water homeostasis critically depends on replenishing water loss, and this compensatory fluid intake, combined with the continuous large diuresis, significantly impairs activities of daily living.[1] No causal treatment for the disease exists, and current approaches for symptomatic treatment aim to ameliorate symptoms with adequate water supply, nonspecific pharmacological therapies and dietary restrictions, as reviewed elsewhere.[2] The diagnosis of congenital NDI is often reached in infancy; clinical findings include polyuria, polydipsia, hypernatremia, plasma hyperosmolality, and hypoosmolar urine.[3] In NDI, there is a normal increase in vasopressin/copeptin plasma concentrations during water deprivation but no sensitivity to exogenous or endogenous AVP.[3] Nephrogenic diabetes insipidus results from impaired vasopressin receptor signaling at target cells or defect aquaporins, and may be acquired (secondary NDI) or congenital (primary NDI).[4] Primary NDI can be caused by mutations in genes encoding aquaporin 2 (AQP2) (autosomal recessive/dominant, 10% of primary NDI cases) or the arginine vasopressin 2 (AVP-V2) receptor (X-linked, 90% of primary NDI cases).[1] Activation of the AVP-V2 receptor promotes water reabsorption through increases in intracellular cyclic adenosine monophosphate (cAMP) level and protein kinase A activity (Figure 1a). No treatment has yet efficiently bypassed an inactive AVP-V2 receptor to raise cAMP in principal cells. *In vitro* studies found a cAMP-independent effect of nitric oxide, L-arginine, and atrial natriuretic peptide to increase the translocation of AQP2 from the cytoplasm to the apical membrane through an increase in cyclic guanosine monophosphate (cGMP).[5] Similarly,

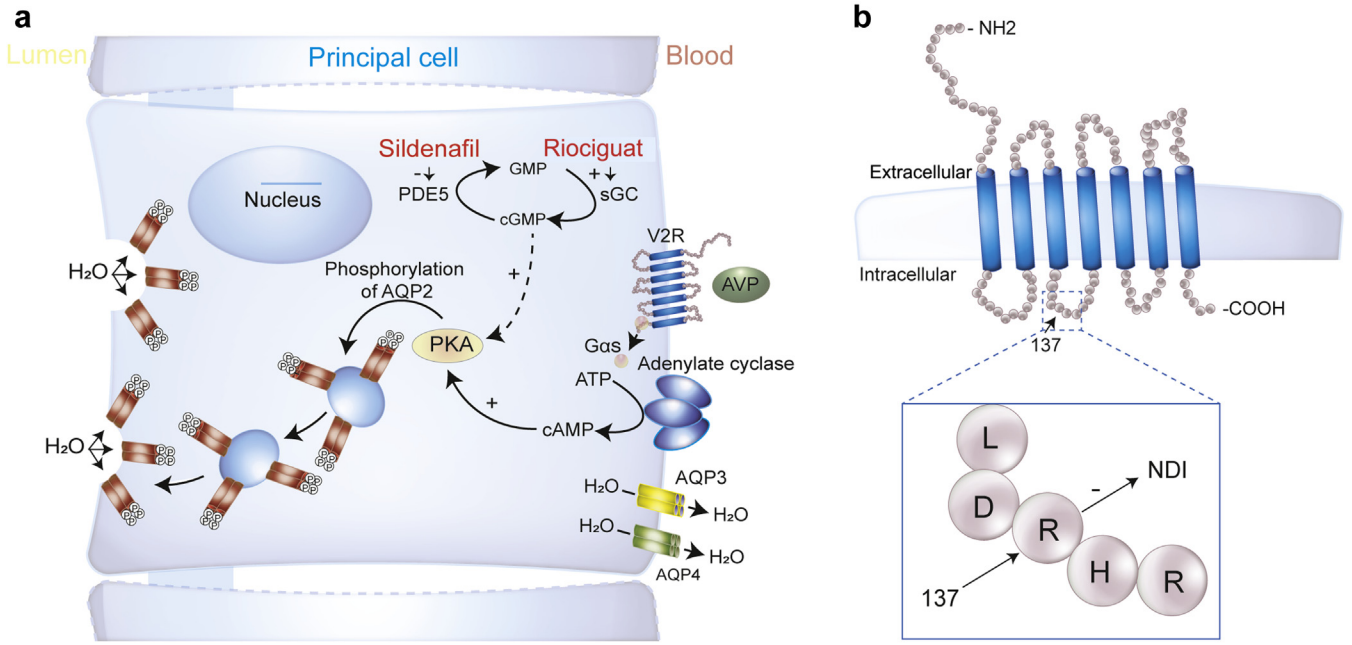

Figure 1. (a) Schematic presentation of the arginine vasopressin (AVP)–mediated trafficking of aquaporin 2 (AQP2) in the principal cells of the collecting ducts and 2 potential treatment strategies for treating nephrogenic diabetes insipidus (NDI). Binding of the AVP to the G protein–coupled transmembrane vasopressin receptor (V2R) located in the basolateral membrane of the principal cell stimulates the release of Gsα, which in turn increases the conversion of adenosine monophosphate (AMP) to cyclic AMP (cAMP). Increased levels of cAMP activate protein kinase A (PKA), which subsequently phosphorylates several residues on the c-terminus of AQP2 and enables trafficking and insertion of AQP2 to the apical membrane as well as facilitates water diffusion along concentration gradients. Water exits the cell by AQP3 and AQP4 constitutively located in the basolateral membrane. Administration of a phosphodiesterase type 5 (PDE5) inhibitor sildenafil or a soluble guanylate cyclase stimulator, riociguat, increases cyclic guanosine monophosphate (cGMP), which potentially could activate PKA and allow trafficking of AQP2. We report the results of these 2 potential treatment strategies for treatment of NDI caused by mutation in the V2R. (b) Illustration of the transmembrane V2R. In the highlighted area, residue 137 is indicated. Mutation results in substitution of glycine for arginine and loss-of-function of the V2R and inappropriate response to AVP stimulation, which results in NDI.

in mouse cortical collecting duct cells, atrial natriuretic peptide, L-arginine, and 8-bromoguanosine 3′5′ cGMP-induced translocation of AQP2.[6] In accordance with these results, the phosphodiesterase type 5 (PDE5) inhibitor sildenafil, which inhibits cGMP degradation, increased the apical redistribution of AQP2 *in vitro* and *in vivo* in rats.[7] In rats with lithium-induced acquired NDI, sildenafil reduced urinary output and increased the medullary expression of AQP2.[8] To our knowledge, the only report of such an effect in humans is a case report with data from a 4.5-year-old boy with X-linked NDI. Administration of sildenafil for 10 days significantly reduced 24-hour urinary output, increased urinary osmolality, and normalized serum osmolality.[9] Thus, it appears that the AVP-V2 receptor pathway can be circumvented through an alternative cGMP-mediated pathway. This may offer a potential treatment strategy in patients with NDI due to an AVP-V2 receptor mutation. We previously identified a new X-linked mutation in the AVP-V2 receptor in male dizygotic twins in our outpatient clinic and characterized their phenotype (Figure 1b).[S1] Here, we present the results of a clinical trial testing the hypothesis that the PDE5-inhibitor sildenafil or the soluble guanylate cyclase (sGC) stimulator riociguat would reduce 24-hour diuresis and water intake and improve urinary concentration ability during water deprivation in 2 male subjects with severe congenital NDI due to a hemizygous mutation in exon 2 of AVPR2 gene: AVPR2:c.C840G;p.R137G (Figure 1a).[S1]

RESULTS

Baseline characteristics are listed in Table 1. Both subjects were obese, with a body mass index of 43.4 and 42.7 kg/m^2, respectively. Blood pressures were within normal range at 127/72 mm Hg and 131/65 mm Hg. Paraclinical values showed slight hypernatremia (146 mmol/l in both subjects), plasma osmolality in the high-normal range (300 mmol/kg), hypokalemia (3.2 and 3.4 mmol/l), and hypomagnesemia (0.62 and 0.66 mmol/l) (Table 1). Both subjects had excessive 24-hour urinary output (10,475 and 11,275 ml/d) with unmeasurable urine sodium concentration and a very low urinary osmolality (97 and 92 mosm/kg), and displayed hyperfiltration as judged by creatinine clearances above 150 ml/min (Table 1).

Table 1. Baseline characteristics

Characteristic	Subject 1	Subject 2	Reference values
Bodyweight (kg)	144.6	142.4	
Height (cm)	182.5	182.5	
Body mass index (kg/cm^2)	43.4	42,8	
SBP (mm Hg)	127	131	
DBP (mm Hg)	72	65	
Pulse (min^{-1})	95	90	
Blood			
Hemoglobin (mmol/l)	9.8	10.2	8.3–10.5
Calcium ion (mmol/l)	1.23	1.25	1.18–1.32
Phosphate (mmol/l)	0.91	0.91	0.71–1.53
Magnesium (mmol/l)	**0.62**	**0.66**	0.71–0.94
Sodium (mmol/l)	**146**	**146**	137–145
Potassium (mmol/l)	**3.2**	**3.4**	3.5–4.4
Albumin (g/l)	45	46	36–50
Creatinine (μmol/l)	68	63	60–105
eGFR (ml/min)	>90	>90	>59
Bicarbonate (mmol/l)	24	25.8	22–26
Alanine transaminase (U/l)	19	16	10–70
Glucose (mmol/l)	7.1	5.6	2.5–11.0
C-reactive protein (mg/l)	8.9	3.9	<6
Osmolality (mosm/kg)	**300**	**300**	280–300
Urine			
Volume (ml/d)	**10,475**	**11,275**	
Creatinine clearance (ml/min)	**154**	**155**	70–150
Protein (g/l)	<0.04	<0.04	
Sodium (mmol/l)	**<20**	**<20**	
Potassium (mmol/l)	7	7	
Osmolality (mosm/kg)	**97**	**92**	300–900

Effect of Sildenafil and Riociguat on Urinary and Plasma Parameters

Figure 2a illustrates the study timeline. Sildenafil and riociguat treatment increased cGMP excretion in 24-hour urine samples during each treatment period compared to baseline samples (Table 2). Plasma cGMP increased but did not show a fold change similar to that in urine. Systolic blood pressure decreased in both subjects during treatment, whereas diastolic blood pressure remained stable (Figure 2b). Treatment with sildenafil did not affect urine output or urine osmolality in the first 4 days; in the last 2 days, 24-hour urinary output declined, but was still at 9 to 10 L/d (Figure 2c). In response to treatment with riociguat, urinary output declined during the first 2 days from very high values (15 L/d) and then increased again to levels above 10 L/d. For both subjects, these fluctuations in urinary output were not accompanied by marked changes in urine osmolality, which was 90 to 100 mosm/kg throughout both observation periods (Fig. 2c). For both subjects, plasma osmolality and plasma sodium concentrations were stable above normal reference range (approximately 300 mosm/kg and 145−150 mmol/l) during both intervention periods (Figure 2d). For both subjects, creatinine clearance declined to near-normal levels in response to sildenafil and in 1 of the subjects in response to riociguat. The other subject had no effect of riociguat on creatinine clearance (Figure 2e). Fractional excretion of K^+ was 10% to 15% and fluctuated within this interval (Figure 2f). Fractional excretion of Na^+ could not be calculated because of unmeasurable urinary Na^+ concentrations.

Effect of Sildenafil and Riociguat on Urinary Concentration Ability

Water deprivation did not reduce urinary output, which was around 600 ml/h in both subjects (Figure 3a), with a corresponding weight loss of 2 to 4 kg during 4 hours of water deprivation (Figure 3a). After the intervention with sildenafil or riociguat, urinary output remained unaltered at values greater than 500 ml/h, as did body weight loss (Figure 3e and i). This was paralleled by a decrease in total body water as judged by bioimpedance (Figure 3b, f, and j). Plasma osmolality increased gradually throughout water deprivation at baseline and after both interventions to maximal levels of 315 mosm/kg; however, urinary osmolality increased only slightly during water deprivation, with no differences before and after the interventions and with maximal levels attained at 130 mosm/kg (Figure 3c, g, and k). Plasma sodium concentrations increased to peak levels of 154 mmol/l, with no difference between baseline and intervention (Figure 3d, h, and l). Plasma levels of AVP increased to levels around 7.8 pmol/l during water deprivation and were not affected by sildenafil or riociguat (Figure 3d, h, and l). Water deprivation tests in both experimental runs and in both probands were terminated after 3 to 4 hours because of plasma sodium levels reaching the predefined safety level of 150 mmol/l.

Side Effects of Sildenafil and Riociguat

The subjects experienced transient upper gastrointestinal symptoms, headaches, and flushing during treatment with sildenafil. Moreover, at the end of the treatment period with sildenafil, both subjects developed mild muscle pain, especially in the lower limbs. Riociguat was generally well accepted, although 1 subject again experienced dyspepsia.

DISCUSSION

The current study investigated the hypothesis that drugs stimulating the cGMP pathway would reduce 24-hour urinary output and improve urinary concentration ability during water deprivation in 2 subjects with congenital NDI due to mutation in the AVPR2 gene. The subjects displayed markedly increased urinary output at approximately 10 to 11 L/d at baseline with low urine osmolality, and although the PDE5-inhibitor sildenafil and the sGC-stimulator riociguat both

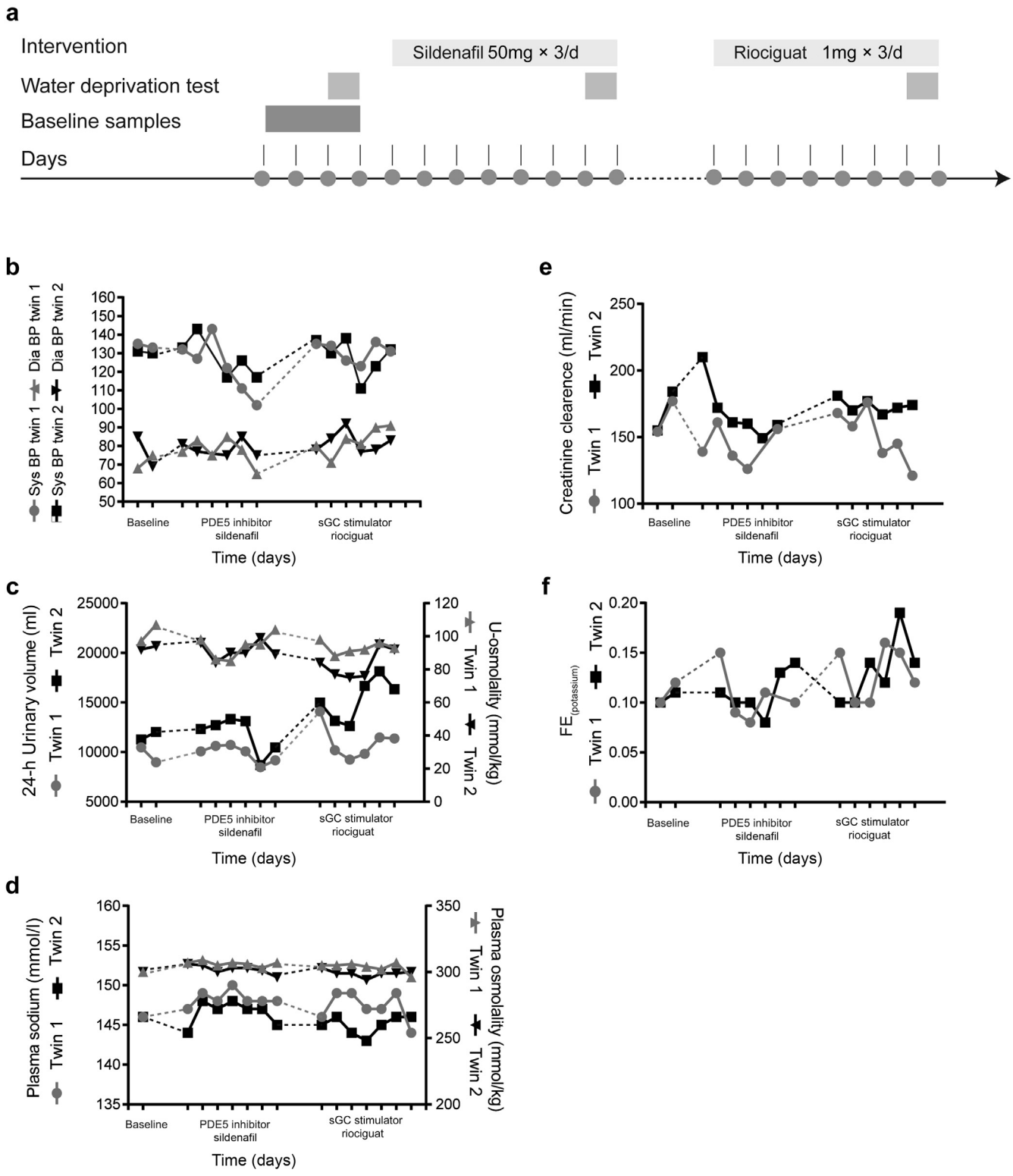

Figure 2. (a) Illustration of the chronological timeline of the experiment. At baseline, the subjects collected 24-hour urinary samples, underwent blood sampling, and performed a water deprivation test. The first part of the study was performed with administration of sildenafil 50 mg 3 times a day for a total of 7 days. The experiment was initiated with hospitalization for 3 days and subsequent daily control in the outpatient clinic at the nephrology department. The last day of treatment, a water deprivation test was carried out. After a washout period, the second part of the experiment was conducted in a similar way, with administration of riociguat 1 mg × 3/d for 7 days. (b–f) Diagram illustrating the effects of treatment with sildenafil or riociguat compared to baseline samples on blood pressure, urinary output and osmolality, plasma sodium and osmolality, creatinine clearance and fractional excretion of potassium. Dia BP, diastolic blood pressure; sGC, soluble guanylate cyclase; Sys BP, systolic blood pressure.

Table 2. Cyclic guanosine monophosphate (cGMP) measurements

	Basis		Sildenafil		Riociguat	
cGMP measurement	Subject 1	Subject 2	Subject 1	Subject 2	Subject 1	Subject 2
Plasma cGMP (pmol/ml)	7.67	5.51	6.52	10.74	6.62	8.29
24-h Urinary cGMP (nmol/d)	964.75	821.95	1179.17	1118.22	1741.71	1234.49
Crude urine cGMP/crea (μmol/g)[a]	0.38	0.41	0.56	0.65	1.03	0.45

[a]Crude urine samples obtained from the last hour during the 3 water deprivation tests.

increased urinary cGMP and lowered blood pressure and hyperfiltration, they had no consistent beneficial effect on urine concentration capacity or urine output, plasma osmolality, or sodium concentration in these subjects. Both treatment strategies were well tolerated without significant side effects.

Experimental data have demonstrated accumulation of AQP2 in the apical membrane of renal collecting ducts in rats with central DI treated with sildenafil, suggesting a vasopressin-independent AQP2 membrane insertion induced by cGMP-mediated signal pathway.[7] However, in mice with knock-out of the AVP-V2 receptors, the only systemic therapy proved to lower diuresis and improve urine concentrating ability and AQP2 trafficking was a cAMP-dependent PGE_2-EP4 receptor agonist[S2], a combination of fluvastatin and secretin,[S3] or metformin.[S4,S5] A functional beneficial effect of sildenafil or other cGMP-dependent agonists measured as increased urine concentration ability has not been demonstrated in male mice with loss-of-function of AVPR2. Thus, although treatment with cGMP seems to be a promising approach to treat NDI patients based on experimental data, validation studies in patients with NDI are lacking because of the rarity of the disease. A study published during the present investigation tested AVP-independent urine concentrating capacity in healthy volunteers treated with sildenafil and showed no effect on urinary osmolality.[S6] These results are in accordance with the present study, but are in contrast to a previous report of a 4.5-year-old boy with congenital NDI also caused by AVPR2 mutation. Here, sildenafil (2 mg/kg per day) reduced urinary output, increased urine osmolality, and normalized plasma sodium and plasma osmolality.[9] These contrasting results could partly be explained by physiological or pharmacodynamic differences between children and adults. It has previously been noted that the effects of standard treatment in NDI may decrease during adolescence.[S7] After termination of the present study, both patients were referred for urodynamic examinations, which revealed a residual volume of 120 to 160 ml after a voided volume of 570 ml and of 530 to 600 ml after a voided volume of 709 ml. It could be speculated that progressive tubulo-interstitial damage resulting from years of high tubular flow rates and high-volume voiding, could lead to less reversibility of polyuria in adults.

Another explanation could be the difference in dosages. In the current study, the sildenafil dosage was approximately 1 mg/kg per day (150 mg daily), whereas the pediatric patient received twice that dosage (2 mg/kg per day).[9] A dosage of 1 mg/kg per day is within the high therapeutic range; in addition, because of side effects and a significant drop in systolic blood pressure, it would not have been feasible to increase dosages in the present study.

Although hydrochlorothiazide and a sodium-restricted diet might attenuate symptoms in NDI,[S8] no causal treatment for the disease exists. Previously, a cross-over study was designed to investigate the effect of sildenafil 100 to 200 mg/d combined with calcitonin for 4 days in addition to standard therapy with hydrochlorothiazide/amiloride and indomethacin in 40 NDI patients 5 to 25 years of age. This study was terminated after completing 4 patients with no effect of sildenafil/calcitonin addition on 24-h urinary volumes compared with standard treatment (unpublished, clinicaltrials.gov: NCT00478335).

To our knowledge, this is the first study to test the effect of riociguat in the context of NDI. From the hypothesis, a better tolerance to water deprivation after treatment with sildenafil and riociguat was predicted; however, this was not observed. Hypernatremia led to termination of the water deprivation tests 1 hour earlier after treatment with sildenafil compared to baseline. In 1 of the subjects, this was partly due to higher start-levels of plasma sodium after sildenafil treatment (148 mmol/l compared to 146 mmol/l at baseline). It seems safe to conclude that there was no beneficial effect of either sildenafil or riociguat on the urinary concentration ability.

A limitation of the present study is the small number of subjects included. However, with the extensive evaluation of each subject and no indication of an effect of either intervention, we believe that the data give proof of concept. The duration of the intervention (7 days) was shorter than that in the previously published pediatric case report (10 days),[9] but given the half-life of sildenafil (3−5 hours) and riociguat (12 hours) and the clear blood pressure responses, it is unlikely that a beneficial effect would appear later than 7 days after initiating treatment. Both proteins PDE5 and sGC have been demonstrated in collecting ducts.[S9−S12]

In summary, the current study found no beneficial effect of sildenafil and riociguat on urinary output or urinary concentration ability in 2 subjects with congenital NDI. It is concluded that in adult patients with congenital inherited NDI, caused by V2 receptor mutation, systemic

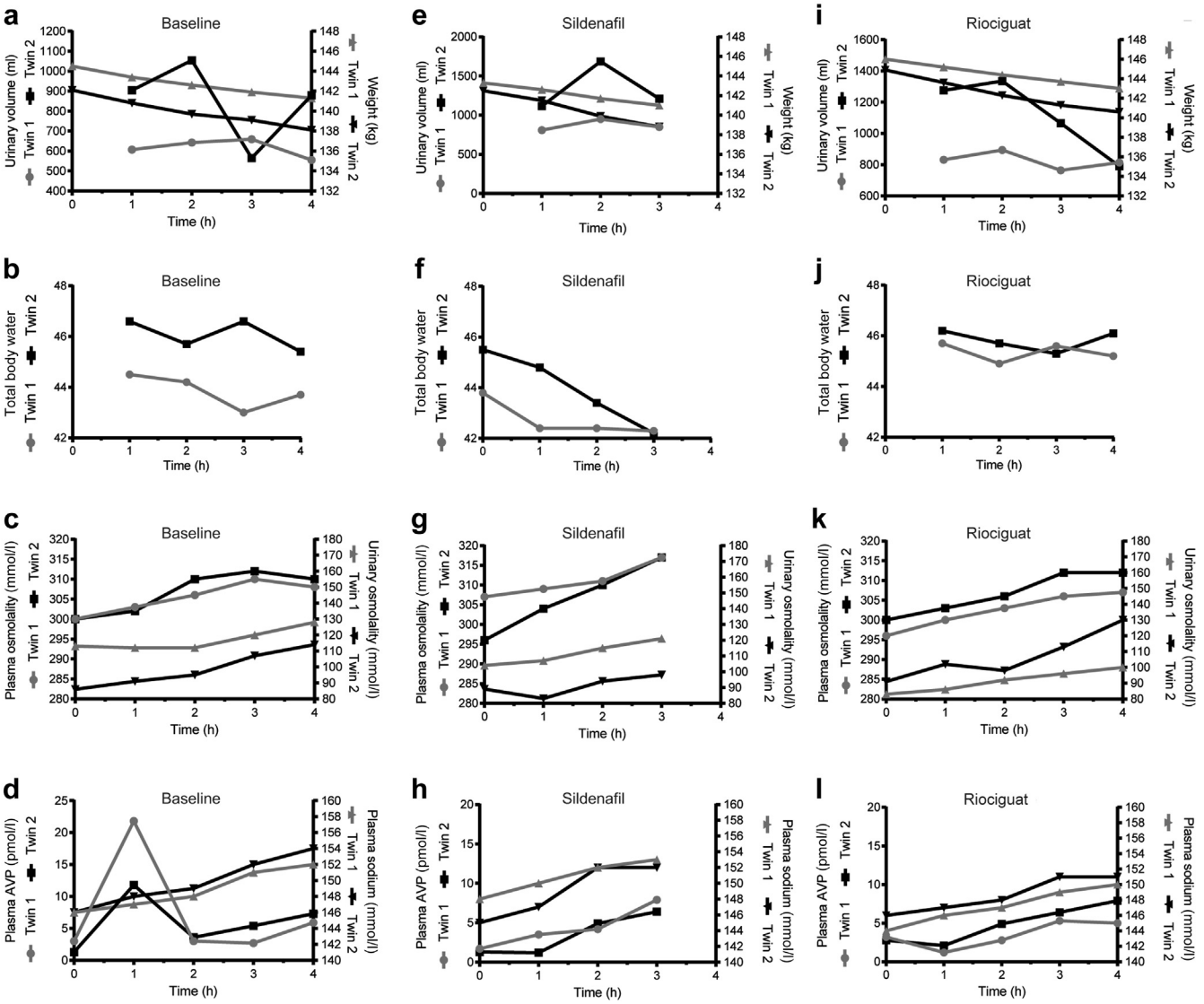

Figure 3. Diagram showing the effects of water deprivation test at baseline (a–d) and during treatment with sildenafil (e–h) and riociguat (i–l). During water deprivation, urinary volume was continuously high, which resulted in a significant weight loss. Plasma osmolality increased, whereas urinary osmolality was unchanged and low. Plasma arginine vasopressin (AVP) concentration increased in parallel to increased plasma sodium concentration. Despite treatment with sildenafil or riociguat, none of the subjects were able to concentrate their urine during water deprivation. Stop-criteria were attained after 3 to 4 hours, and water deprivation was abolished.

pharmacological stimulation of the cGMP pathway, does not rescue the phenotype or improve urine concentration ability.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

The authors thank the patients for their participation in the study. The authors are appreciative for the financial support from The Region of Southern Denmark.

AUTHOR CONTRIBUTIONS

GH, CB, HD, and BLJ developed the study design and hypothesis. GH and LM conducted the experiments. LM drafted the manuscript. GH prepared figures. GH, CB, HD, and BLJ critically revised the manuscript. All authors approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

REFERENCES

1. Bockenhauer D, Bichet DG. Pathophysiology, diagnosis and management of nephrogenic diabetes insipidus. *Nat Rev Nephrol*. 2015;11:576–588.
2. Milano S, Carmosino M, Gerbino A, et al. Hereditary nephrogenic diabetes insipidus: pathophysiology and possible treatment. An update. *Int J Mol Sci*. 2017;18.
3. Christ-Crain M, Bichet DG, Fenske WK, et al. Diabetes insipidus. *Nat Rev Dis Primers*. 2019;5:54.
4. Moeller HB, Rittig S, Fenton RA. Nephrogenic diabetes insipidus: essential insights into the molecular background and potential therapies for treatment. *Endocr Rev*. 2013;34:278–301.
5. Bouley R, Breton S, Sun T, et al. Nitric oxide and atrial natriuretic factor stimulate cGMP-dependent membrane insertion of aquaporin 2 in renal epithelial cells. *J Clin Invest*. 2000;106:1115–1126.
6. Boone M, Kortenoeven M, Robben JH, et al. Effect of the cGMP pathway on AQP2 expression and translocation: potential implications for nephrogenic diabetes insipidus. *Nephrol Dial Transplant*. 2010;25:48–54.
7. Bouley R, Pastor-Soler N, Cohen O, et al. Stimulation of AQP2 membrane insertion in renal epithelial cells in vitro and in vivo by the cGMP phosphodiesterase inhibitor sildenafil citrate (Viagra). *Am J Physiol Renal Physiol*. 2005;288:F1103–F1112.
8. Sanches TR, Volpini RA, Massola Shimizu MH, et al. Sildenafil reduces polyuria in rats with lithium-induced NDI. *Am J Physiol Renal Physiol*. 2012;302:F216–F225.
9. Assadi F, Ghane Sharbaf F. Sildenafil for the treatment of congenital nephrogenic diabetes insipidus. *Am J Nephrol*. 2015;42:65–69.

Fibrillary Glomerulonephritis Is Associated With HLA-DR7 and HLA-B35 Antigens

Nicole K. Andeen[1], Kelly D. Smith[2], Elena-Rodica Vasilescu[3] and Ibrahim Batal[3]

[1]Department of Pathology, Oregon Health & Science University, Portland, Oregon, USA; [2]Department of Pathology, University of Washington, Seattle, Washington, USA; and [3]Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, New York, USA

Correspondence: Ibrahim Batal, Department of Pathology and Cell Biology, Renal Division, Columbia University Irving Medical Center, 630 West 168th street, VC14-238, New York, New York 10032, USA. E-mail: ib2349@columbia.edu

Received 9 March 2020; revised 17 April 2020; accepted 4 May 2020; published online 20 May 2020

Kidney Int Rep (2020) **5,** 1325–1327; https://doi.org/10.1016/j.ekir.2020.05.010

Fibrillary glomerulonephritis (FGN) is a rare immune-mediated glomerulonephritis with an incompletely understood pathogenesis, characterized by glomerular deposits of randomly oriented fibrils (12−24 nm in diameter). The majority of FGN cases are Congo red negative and composed of polyclonal IgG.[1−3] DNAJB9 is a novel biomarker for FGN; expression of DNAJB9 in glomeruli is highly sensitive and specific for FGN,[4,5] and elevated serum levels of DNAJB9 have been observed in patients with FGN.[6] Because of the association with hepatitis C virus and autoimmune diseases, FGN has been linked to chronic immune stimulation in some patients. Most commonly, FGN is encountered in Caucasians.[1] Although only exceptional cases of familial FGN have been reported,[1,7−9] this is at least partially related to the rarity of FGN. In the United States, FGN is diagnosed in 1% of native kidney biopsy specimens compared with approximately 7% showing IgA nephropathy.[S1] Taken together, we hypothesized that genetic background plays a role in the pathogenesis of FGN. Because human leukocyte antigens (HLAs) have emerged as important inherited risk factors in most immune-mediated renal diseases,[S2] we examined the association of FGN with different HLAs.

We retrospectively identified a multi-institutional cohort of patients with FGN and available HLA typing (n = 26; Columbia University [n = 18], Oregon Health & Science University [n = 6], University of Washington [n = 2]). The cases comprised transplant recipients with end-stage kidney disease secondary to FGN (n = 23), *de novo* FGN in allograft (n = 2), and donor with FGN (n = 1). The HLA serotyping in this cohort was compared to internal controls from deceased kidney donors (DKDs, n = 96) and external controls of US residents of European descent from the National Marrow Donor Program (n = 15,740), as described in Supplementary Methods.

We initially identified the most frequent class I and class II antigens in FGN patients (Table 1). These