DOI 10.1515/jpem-2013-0097 — J Pediatr Endocr Met 2013; aop

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Novel *AQP2* mutation causing congenital nephrogenic diabetes insipidus: challenges in management during infancy

Abstract: Congenital nephrogenic diabetes insipidus (NDI) is a rare inherited disorder, mostly caused by *AVPR2* mutations. Less than 10% of cases are due to mutations in the *aquaporin-2* (*AQP2*) gene. Diagnosis and management of this condition remain challenging especially during infancy. Here, we report two unrelated patients, a 6-month-old Thai boy and a 5-year-old Emirati girl, with a history of failure to thrive, chronic fever, polydipsia, and polyuria presented in early infancy. The results of water deprivation test were compatible with a diagnosis of NDI. The entire coding regions of the *AVPR2* and *AQP2* gene were amplified by polymerase chain reaction and sequenced. Patient 1 was homozygous for a novel missense *AQP2* mutation p.G96E, inherited from both parents. Patient 2 harbored a previously described homozygous p.T126M mutation in the *AQP2* gene. Both patients were treated with a combination of thiazide diuretics and amiloride. Patient 1 developed paradoxical hyponatremia and severe dehydration 2 weeks after medical treatment began. In conclusion, we report a novel mutation of the *AQP2* gene and highlight an important role of genetic testing for definite diagnosis. Vigilant monitoring of the fluid status and electrolytes after beginning the therapy is mandatory in infants with NDI.

Keywords: Aquaporin-2 (*AQP2*); congenital nephrogenic diabetes insipidus; mutation; novel.

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Introduction

Nephrogenic diabetes insipidus (NDI) is an inherited or acquired disorder where renal collecting ducts cannot concentrate urine in response to arginine vasopressin (AVP). Patients with congenital NDI usually present within the first year of life with polyuria, polydipsia, vomiting, anorexia, fever, and failure to thrive (1). Most of these cases are inherited in an X-linked recessive pattern, caused by mutations in the *arginine vasopressin receptor 2* (*AVPR2*) gene (1, 2). Less than 10% of cases have autosomal recessive mutations in the gene encoded for *aquaporin 2* (*AQP2*), which is the AVP-dependent water channel of the collecting duct (3). A very rare autosomal dominant form has also been reported (4).

Management of congenital NDI consists of adequate supply of fluid intake and renal solute reduction by a low-sodium diet to decrease urine output, combined with hydrochlorothiazide (HCTZ) and amiloride or renal prostaglandin synthetase inhibitors (1). These therapeutic approaches usually do not reduce urine output entirely to normal. Thus, increased water intake is generally still needed, especially in tropical area. The management of congenital NDI is complex in infants who cannot self-regulate oral fluid intake. A few previous studies reported cases with congenital NDI that was complicated by water intoxication, and severe hyponatremia secondary to excessive water intake, and the initiation of medical treatment (5–7).

Here, we describe two unrelated Asian patients with NDI presented in early infancy and identified their *AQP2* mutations, one of which is novel. Interestingly, one patient developed severe hyponatremia and dehydration once medical treatment began.

Case reports

Patient 1

A 6-month-old boy presented with polydipsia, polyuria, and recurrent fever. He was born at term with a birth weight of 3100 g. No other family members had DI symptoms. His paternal and maternal grandmothers were siblings (Figure 1). His length was 60 cm (third percentile), weight was 4430 g (third percentile), and head circumference was 39 cm (third percentile). Results of other physical examinations were unremarkable. Initial laboratory evaluation included serum Na 147 mmol/L, K 4.1 mmol/L, Ca 10.6 mg/dL, blood urea nitrogen (BUN) 19 mmol/L, and creatinine (Cr) 0.26 mg/dL. Serum and urine osmolalities were 306 and 61 mOsm/L, respectively. Urine osmolality did not increase after administration of subcutaneous 1-desamino-8-D-arginine vasopressin (dDAVP) at the end of water deprivation test (Table 1). Twenty-four-hour urine collection demonstrated normal tubular function. Ultrasonography (USG) result of the urinary system was normal. Brain magnetic resonance imaging (MRI) showed the small size of the pituitary gland and pituitary stalk accompanied by absent posterior bright spot. His anterior pituitary hormones were normal. Treatment with a combination of amiloride 0.2 mg/kg per day and hydrochlorothiazide 2 mg/kg per day improved his polyuria from 2.6–3.4 to 0.7–1.0 L/m^2 per day. Two weeks later at the follow-up visit, he was clinically dehydrated and weighed 4250 g (pre-discharge weight 4570 g). Electrolytes showed that serum Na was 113; K, 6.5; Cl, 80; HCO_3, 17 mmol/L; BUN, 45 mmol/L; Cr, 0.23 mg/dL; and Ca, 10.9 mg/dL. Urine electrolytes revealed Na was 26; K, 21; and Cl 24, mmol/L.

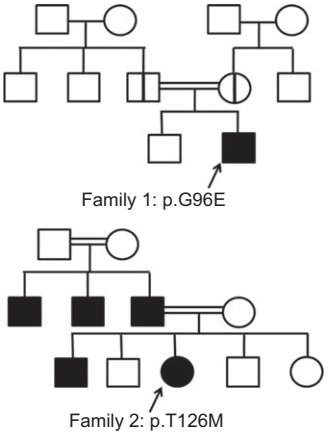

Figure 1 Pedigrees of families 1 and 2 with *AQP2* mutations. All affected individuals are in black symbols, and probands are indicated by arrows.

Table 1 Results of water deprivation tests.

	Patient 1	Patient 2
Baseline		
Serum sodium, mmol/L	147	142
Serum osmolality, mOsm/L	306	289
Urine osmolality, mOsm/L	61	39
After water deprivation		
Serum sodium, mmol/L	149	152
Serum osmolality, mOsm/L	313	324
Urine osmolality, mOsm/L	66	61
After dDAVP, 0.025 μg/kg, subcutaneous		
Urine osmolality, mOsm/L	61	61

Fluids were withheld after 8 a.m.; body weight, urine output, vital sign, serum and urine sodium and osmolality were measured hourly. dDAVP was given when weight fell by 5% or serum osmolality exceeded 300 mOsm/L.

The medical treatment was then stopped. The patient received intravenous fluid to correct dehydration and electrolyte imbalance. A week later, hydrochlorothiazide was restarted when he developed polyuria and hypernatremia, titrated to 2 mg/kg per day, combined with indomethacin 1 mg/kg per day. At the 2 week follow-up visit, he had no clinical signs of dehydration. His urine output was 1.6–2.0 L/m^2 per day, and serum electrolyte and Cr levels were normal. At his last visit (age 1 year), his weight and height were still below the third percentile and he had delayed developmental milestones. He still had mild polyuria (urine output 2–3 L/m^2 per day), but normal electrolyte levels and normal hydration status.

Patient 2

A 5-year-old girl presented at 5 months of age with recurrent vomiting and failure to thrive. She developed hypernatremic dehydration with serum sodium levels up to 162 mmol/L during repeated hospital admissions for febrile illnesses. She was noted to be irritable and preferred drinking water rather than milk. She also had a history of polyuria. At the age of 13 months, she was diagnosed with diabetes insipidus and was prescribed hydrochlorothiazide with no marked effect on her urine output. She is the middle child of five siblings. Her parents are first-degree cousins (Figure 1). Her father and two of her paternal uncles were diagnosed with NDI during childhood. The father is 42 years old and had a similar history to the index case as he presented as a child with polyuria and polydipsia. Currently, he drinks 7–9 L/day and passes urine excessively. He has never been on any medications for his DI. A water deprivation test was performed in patient 2 at age

5 years which revealed elevated plasma osmolality with persisting inappropriately low urine osmolality (Table 1). Administration of desmopressin showed no increase in urine osmolality. Her AVP levels were 18.97 pmol/L (normal range <13 pmol/L) at a serum osmolality of 289 mOsm/L. Her USG of urinary tract showed normal kidneys and uretero-pelvicalyceal system. A water deprivation test on her father was also compatible with NDI. The father was found to have impaired kidney function from a Tc^{99} MAG3 kidney scan, and USG showed marked pelvicalyceal dilatation of the right kidney. The mother denied any symptoms of DI. Our patient was treated with amiloride (0.3 mg/kg per day) and hydrochlorothiazide (3 mg/kg per day) with modest improvement of her symptoms. Her growth parameters remained normal.

Methods

Polymerase chain reaction and DNA sequencing

With informed consent, genomic DNA was extracted from peripheral leukocytes. All coding regions of the *AQP2* and *AVPR2* genes and the exon-intron splicing junction boundaries were amplified by polymerase chain reaction, using the primers and conditions as previously described (8, 9), and sequenced. For a novel missense mutation, restriction enzyme digestion was used to confirm its presence in the patient and parents as well as to screen in 100 control chromosomes from unaffected ethnic-matched individuals, which would detect < 5% polymorphism with 95% power (10).

Results

Direct sequencing revealed patient 1 was homozygous for a novel missense *AQP2* mutation located in exon 1, a guanine-to-adenine substitution at nucleotide position 287 (c.287G>A), resulting in a glycine-to-glutamic acid substitution at codon 96 (p.G96E) (AQP2 protein reference sequence NP_000477.1) (Figure 2A). This mutation has never been previously described and was not detected in 100 ethnic-matched unaffected control chromosomes (Figure 3). Restriction enzyme digestion analysis of exon 1 of parental genomic DNA revealed that the parents were heterozygous carriers of the mutation and clinically asymptomatic. Patient 2 and her father carried a previously described homozygous missense *AQP2* mutation, a cytosine-to-thymine substitution at nucleotide position 377 (c.377C>T), resulting in a threonine-to-methionine replacement at codon 126 (p.T126M) (Figure 2B). The mother's DNA was not available.

Discussion

We described two unrelated patients with congenital NDI and identified one previously described and one novel *AQP2* mutations. Patient 1 harbored a novel missense mutation, p.G96E. This variant has not been reported to be a polymorphism in NCBI SNP or Ensembl databases, and it was not detected in 100 ethnic-matched control chromosomes, supporting it as a disease-causing mutation. The p.G96E is located in the third transmembrane domain of *AQP2*. Mutations in this region leading to protein misfolding, and thus misrouting of *AQP2* transport to the plasma membrane, have been reported (11, 12). Patient 2 presented with p.T126M mutation, which was first reported in two siblings from Sri Lanka. Previous in vitro study showed that the p.T126M mutant protein was impaired in its transport to the plasma membrane when expressed in *Xenopus* oocytes (13).

The MRI study in Patient 1 revealed the small size of the pituitary gland and stalk accompanied by absent

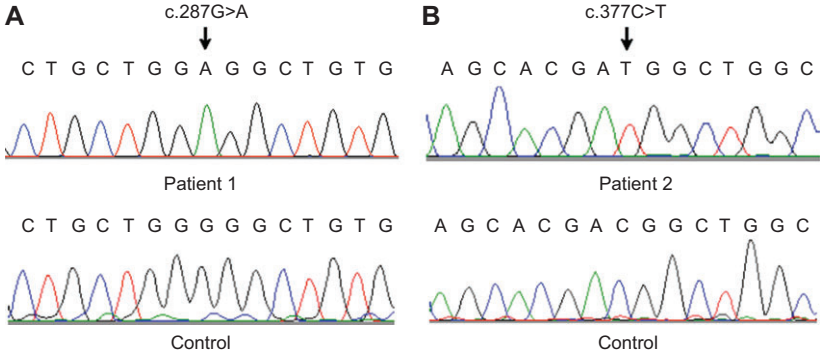

Figure 2 Mutation analysis by direct DNA sequencing of the *AQP2* gene. (A) Patient 1 carrying a homozygous novel missense p.G96E mutation (c.287G>A). (B) Patient 2 carrying a homozygous p.T126M mutation (c.377C>T). Each mutation is indicated by an arrow.

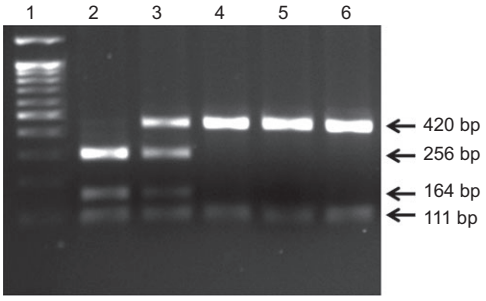

Figure 3 Restriction enzyme digestion analysis. Lane 1: 100-bp marker. Lane 2: Patient 1 (p.G96E). Lane 3: Father (heterozygous carrier). Lanes 4–6: normal controls. Restriction enzyme analysis of the polymerase chain reaction products showing the c.287G>A creating an additional cleavage site for the restriction enzyme *Bmp*I resulting in 256- and 164-bp products. The wild-type alleles are digested resulting in 420-bp bands.

posterior bright spot, which could mislead to the diagnosis of central DI. This observation has been made previously and may be explained by depleted vasopressin in neurosecretory granules in untreated NDI (14, 15). Thus, an absence of bright spot in DI patients should be carefully interpreted and correlated with other clinical and laboratory findings.

Management of patients with congenital NDI consists of adequate supply of fluid, sodium restriction, combined with thiazide and/or amiloride diuretics as the current first-line drug (1). In young children, the combination of HCTZ and indomethacin has been commonly used because these patients often have nausea from amiloride. Selective inhibitors of cyclooxygenase-2 can be used in patients who cannot tolerate indomethacin. Thiazide acts by inhibiting sodium reabsorption in the distal tubule by blocking the NaCl co-transporter, leading to hypovolemia resulting in sodium and water reabsorption in the proximal tubules. HCTZ may also induce *AQP2* up-regulation via an AVP-independent mechanism (16). Amiloride acts by inhibiting epithelial sodium channels, resulting in decreased sodium reabsorption and hypovolemia. Prostaglandin synthetase inhibitors cause a reduction in glomerular filtration rate, leading to increased sodium and water reabsorption in proximal tubules, and probably act by inhibiting the retrieval of *AQP2* from the apical membrane of the principal cells (1).

Most missense mutations causing X-linked NDI or autosomal-recessive NDI typically lead to aberrant folding of mutant proteins inside the endoplasmic reticulum. Thus, the aim of current and future research in novel therapeutic modalities in congenital NDI is to find pharmacological chaperones to partially rescue the cell-surface expression and functional activity of some misfolded mutant proteins (17, 18). Therefore, understanding the mechanism of specific mutations would be of high therapeutic importance in the future.

Both patients were initially treated with HCTZ and amiloride with modest improvement of their symptoms. There are few previously reported cases with congenital NDI that were complicated by water intoxication, and severe hyponatremia secondary to excessive water intake, and the initiation of HCTZ (5–7). By contrast, our Patient 1 developed hyponatremic dehydration after beginning the medical treatment for 2 weeks. This could be explained by inadequate water intake and natriuresis due to the effects of HCTZ and amiloride. The supportive evidences are that the patient had weight loss, prerenal azotemia, hypochloremia and relatively high urine sodium concentration. To our knowledge, this finding has never been reported. To avoid this problem, the parents or primary caregivers should be advised to strictly measure fluid intake/output, weigh the child daily, observe for signs of dehydration and of drug overdose (headache, confusion, nausea), and know when to have serum Na levels checked.

Our cases highlight the need for meticulous monitoring of hydration status, fluid balance, weight, serum, and urine electrolytes in NDI patients after beginning the medical treatment. This study also expands the genotypic spectrum of *AQP2* mutations and emphasizes an important role of genetic testing for definite diagnosis.

Acknowledgments: We are grateful to Ms. Siraprapa Tongkobpetch and Mr. Chalurmpon Srichomthong for their technical assistance. This study was supported by the Thailand Research Fund (to V.S. and T.S.), National Research University Project of Thailand, Office of the Higher Education Commission (HR1163A), and Ratchadapisek Sompotch Fund (RES560530177HR), Faculty of Medicine, Chulalongkorn University.

Conflict of interest statement

Disclosure statement: The authors have no conflict of interests.

Received March 13, 2013; accepted July 15, 2013

References

1. Wesche D, Deen PM, Knoers NV. Congenital nephrogenic diabetes insipidus: the current state of affairs. Pediatr Nephrol 2012;27:2183–204.
2. Babey M, Kopp P, Robertson GL. Familial forms of diabetes insipidus: clinical and molecular characteristics. Nat Rev Endocrinol 2011;7:701–14.
3. Deen PM, Verdijk MA, Knoers NV, Wieringa B, Monnens LA, et al. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. Science 1994;264:92–5.
4. Robben JH, Knoers NV, Deen PM. Cell biological aspects of the vasopressin type-2 receptor and aquaporin 2 water channel in nephrogenic diabetes insipidus. Am J Physiol Renal Physiol 2006;291:F257–70.
5. Onozaki A, Suzuki H, Imamura N, Katoh T, Watanabe T. Congenital nephrogenic diabetes insipidus with marked hyponatremia. Clin Exp Nephrol 2001;5:265–7.
6. Boussemart T, Nsota J, Martin-Coignard D, Champion G. Nephrogenic diabetes insipidus: treat with caution. Pediatr Nephrol 2009;24:1761–3.
7. Anton-Gamero M, Garcia-Martinez E, Fernandez-Ramos J, Rodríguez-Salas M, Gil-Campos M. Nephrogenic diabetes insipidus: the key element of paradoxical hyponatremia. Pediatr Nephrol 2009;24:2277–8.
8. Sahakitrungruang T, Wacharasindhu S, Sinthuwiwat T, Suporn-silchai V, Suphapeetiporn K, et al. Identification of two novel aquaporin-2 mutations in a Thai girl with congenital nephrogenic diabetes insipidus. Endocrine 2008;33:210–4.
9. Sahakitrungruang T, Tee MK, Rattanachartnarong N, Shotelersuk V, Suphapeetiporn K, et al. Functional characterization of vasopressin receptor 2 mutations causing partial and complete congenital nephrogenic diabetes insipidus in Thai families. Horm Res Paediatr 2010;73:349–54.
10. Collins JS, Schwartz CE. Detecting polymorphisms and mutations in candidate genes. Am J Hum Genet 2002;71: 1251–2.
11. Sasaki S. Aquaporin 2: from its discovery to molecular structure and medical implications. Mol Aspects Med 2012;33:535–46.
12. Lin SH, Bichet DG, Sasaki S, Kuwahara M, Arthus MF, et al. Two novel aquaporin-2 mutations responsible for congenital nephrogenic diabetes insipidus in Chinese families. J Clin Endocrinol Metab 2002;87:2694–700.
13. Mulders SM, Knoers NV, Van Lieburg AF, Monnens LA, Leumann E, et al. New mutations in the AQP2 gene in nephrogenic diabetes insipidus resulting in functional but misrouted water channels. J Am Soc Nephrol 1997;8:242–8.
14. Sato N, Ishizaka H, Yagi H, Matsumoto M, Endo K. Posterior lobe of the pituitary in diabetes insipidus: dynamic MR imaging. Radiology 1993;186:357–60.
15. Ranadive SA, Ersoy B, Favre H, Cheung CC, Rosenthal SM, et al. Identification, characterization and rescue of a novel vasopressin-2 receptor mutation causing nephrogenic diabetes insipidus. Clin Endocrinol (Oxf) 2009;71:388–93.
16. Kim GH, Lee JW, Oh YK, Chang HR, Joo KW, et al. Antidiuretic effect of hydrochlorothiazide in lithium-induced nephrogenic diabetes insipidus is associated with upregulation of aquaporin-2, Na-Cl co-transporter, and epithelial sodium channel. J Am Soc Nephrol 2004;15:2836–43.
17. Morello JP, Salahpour A, Laperriere A, Bernier V, Arthus MF, et al. Pharmacological chaperones rescue cell surface expression and function of misfolded V2 vasopressin receptor mutants. J Clin Invest 2000;105:887–95.
18. Yang B, Zhao D, Verkman AS. Hsp90 inhibitor partially corrects nephrogenic diabetes insipidus in a conditional knock-in mouse model of aquaporin-2 mutation. FASEB J 2009;23:503–12.