NOTE

A Case of a Novel Mutant Vasopressin Receptor-Dependent Nephrogenic Diabetes Insipidus with Bilateral Non-Obstructive Hydronephrosis in a Middle Aged Man — Differentiation from Aquaporin-Dependent Nephrogenic Diabetes Insipidus by the Response of Factor VIII and von Willebrand Factor to 1-Deamino-8-D-Arginine Vasopressin Administration —

MASASHI MIYAKOSHI, KYUZI KAMOI, SHINICHI UCHIDA* AND SEI SASAKI*

The Department of Internal Medicine, Division of Endocrine and Metabolism, Nagaoka Red Cross Hospital, Nagaoka Niigata 940-2085, Japan

**The Department of Homeostasis Medicine and Nephrology, Tokyo Medical and Dental University, Tokyo 113-8519, Japan*

Abstract. We describe a case of a novel mutant vasopressin 2 receptor (V2R)-dependent nephrogenic diabetes insipidus (NDI) with bilateral non-obstructive hydronephrosis in a middle aged man. This could be distinguished from aquaporin 2 (AQP2)-dependent NDI by the response of factor VIII and von Willebrand factor (vWF) to 1-deamino-8-D-arginine vasopressin (DDAVP) administration. A 47-year-old man was admitted to hospital because of polyuria, which had been present from infancy and was suspected of causing non-obstructive hydronephrosis. His mother's father, the older brother of his mother and his second daughter also all had polyuria. Sodium concentration, osmolality and vasopressin in blood were high, while sodium concentration and osmolality in urine were low. There were no changes in urine osmolality, factor VIII and vWF in response to DDAVP infusion. Neither was heart rate, diastolic blood pressure nor facial flushing affected. These findings suggested this case was V2R-dependent NDI rather than AQP2-dependent NDI. Molecular genetic analysis demonstrated that the patient had a V2R missense mutation involving a substitution of cysteine for arginine at position 104 (R104C) located in the first extracellular loop of the V2R. It was also found that the patient's mother and his second daughter were heterozygous for this R104C mutation.

Key words: AVPR gene, Nephrogenic diabetes insipidus, DDAVP, factor VIII and von Willebrand factor (vWF)
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Nephrogenic diabetes insipidus (NDI) is a kidney disorder characterized by the kidney's inability to respond to the antidiuretic hormone, arginine vasopressin (AVP) [1]. Up to 90% of inherited NDI is caused by mutations of the AVP receptor 2 gene (V2R). The remaining cases are the result of mutations of the aquaporin 2 (AQP2) gene or other unknown disturbances [1]. Although each type of inherited

NDI has a different genetic origin, the symptoms are identical [1–8].

How do we differentiate these types of disorders by clinical examination? Administering 1-deamino-8D-arginine vasopressin (DDAVP), and then measuring responses occurring outside the kidney, such as changes in heart rate, diastolic blood pressure, facial flushing, tissue-type plasminogen activator antigen (tPA) and factor VIII and von Willebrand factor (vWF) [1–8], is one way that clinicians have of distinguishing between NDI caused by mutant V2R genes and by mutant AQP2 genes. The patient with NDI caused by mutant AQP2 genes will show responses to DDAVP that take place outside the kidney, while the patient

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Correspondence to: Dr. Kyuzi KAMOI, the Department of Internal Medicine, Nagaoka Red Cross Hospital, 297-1 Terajima-machi, Nagaoka, Niigata, 940-2085, Japan

whose NDI is the result of mutated V2R genes will not [1–8].

Recently, Inaba *et al.* reported a patient with NDI caused by a novel mutant VR2 with substitution of cysteine for arginine-104 (R104C) diagnosed at middle age [9]. Here, we report a case of the same mutant R104C-dependent NDI distinguished from AQP2-dependent NDI by responses of factor VIII and vWF to DDAVP in a middle-aged Japanese man.

Case Report and Methods

A 47-year-old man was admitted to hospital because of polyuria (4000 ml/day) pointed out by an urologist. Although the patient had had polyuria and polydipsia from infancy, he had no awareness or symptoms of disease and no medication until admission. At the age of 47 years, hypercreatininemia (serum creatinine 1.4 mg/dl) was discovered on regular check-up, after which hydronephrosis was diagnosed by abdominal echogram as a result of examination for polyuria. His

mother's elderly brother aged 85 years, as well as his mother's father who died at the age of 80 years, also had polyuria and polydipsia. His mother aged 75 years, and his first daughter aged 15 years had no symptoms of polyuria and polydipsia, while his second daughter aged 13 years had a mild degree of polyuria and polydipsia (Fig. 1).

On admission, height was measured as 171 cm and weight 68.3 kg. Blood pressure was 134/80 mmHg, and pulse rate was regular at 80/min. Body temperature was 36.7 °C. His tongue was dry.

General laboratory data showed sodium concentration, urea nitrogen, creatinine and osmolality in blood to be 148 mEq/l, 8.2 mg/dl, 1.0 mg/dl and 295 mosmol/kg, respectively, while sodium concentration and osmolality in urine were 28 mEq/l and 94 mosmol/kg, respectively. Urinary volume was 4400 to 8000 ml/day and intake-volume 3100 to 3800 ml/day. Pyuria was present. CT scanning of the abdomen showed that renal pelvises and urinary tracts were dilated without any organic obstruction and that the bladder wall was thickened.

Fig. 1. Pedigree of NDI relatives. Circles, female; squares, men. Roman numerals, the generation. Hatched bar and black symbols, subjects with polyuria and polydipsia. Black symbol, subject for the mutation; circles with a central dot, subject is heterozygotic for one of the mutations. A slash (/) through a symbol indicates that the subject is deceased. The arrow indicates the index case.

Water deprivation testing resulted in no decrease of urine volume at a 1.6 % decrease of body weight with plasma and urine osmolality being 300 and 283 mosmol/kg, respectively. Plasma AVP concentrations were 6 to 13 pg/ml. Urinary volume and osmolality did not respond to nasal administration of 10 µg of DDAVP.

The DDAVP infusion test was performed following the method of Hochberg *et al.* [8]. Before DDAVP infusion, a water-load of 600 ml/m² was given orally to suppress endogenous AVP. Basal urine was collected for an hour. Starting at time zero, DDAVP was infused over 10 min at a dose of 0.3 µg/kg BW. Urine and blood were collected at 0, 60, and 120 min. Plasma and urine osmolality, factor VIII and vWF factor were measured. Basal levels of factor VIII and vWF factor were 160% and 110%, respectively. Urine osmolality, factor VIII (160%) and vWF (108%), as well as heart rate, diastolic blood pressure and facial flushing after the infusion, failed to respond to DDAVP infusion.

These findings indicated that the patient had V2R-dependent NDI rather than acquired NDI or AQP2-dependent NDI. Oral administration of hydrochlorothiazide 50 mg/day and triamterene 100 mg/day decreased urinary volume (3000 ml/day) with increased osmolality (123 mosmol/kg).

Mutation analysis of the V2R gene was performed. Informed consent for DNA analysis was obtained from the patient and his family, and was approved by the institutional review board. Genomic DNA was prepared from peripheral leukocytes by standard methods. DNA sequencing was performed as previously described (10). First, the gene was amplified by PCR with oligonucleotide primers (forward: 5'-TGACCA TCCCCTCTCAATCTTC-3'; reverse: 5'-TCCCTCT TTCCTGCACTCCT-3'). Then, nested PCR was performed using the following primers: 5'-CCCAGGAC TGGCCATACTG-3' and 5'-CCAGCTCAGTGAGCT GAC-3'. The 2 kb PCR product, which covered the exons, the introns, about 140 bp of the 5'-translated region, and about 220 bp of the 3'-untranslated region of the VR2 gene, was obtained. A total of 12 different primers (sense and antisense) were needed for direct sequencing of the PCR product. Final nested PCR was done with primer 5'-CGGAGCTGGCGCTGCT-3'. Direct sequencing of the PCR product was performed on both strands by an automated DNA sequencer model 373S (Applied Biosystems Japan, Chiba, Japan) using the PRISM dye termination kit (Applied Bio-

systems, Chiba, Japan) according to the manufacturer's instructions. The analysis identified a C to T transition at nucleotide position + 310 in exon 2, leading to a predicted change of arginine at codon 104 (CGC) to a cysteine (TGC) (Fig. 2). This mutation was the same V2R missense mutation (R104C) located in the first extracellular loop of the V2R as reported by Inaba *et al.* [9]. Furthermore, the patient's mother and his second daughter aged 13 years were heterozygotic for this mutation (Fig. 2).

Discussion

This patient was diagnosed with NDI at middle age, although he had had a history of polyuria and polydipsia from infancy. One reason for the delayed recognition of NDI was that he had no awareness of being sick in this respect prior to admission to hospital, since the male members of his family had the same signs and symptoms as he did but without any detrimental effects and no impact on their daily activities.

The main symptoms in patients with hereditary NDI

Fig. 2. Direct sequence analysis of AVP receptor 2 (V2R) gene. PCR product was directly sequenced as described in Methods. Direct sequencing of the V2R gene of the patient revealed a missense mutation in the exon 2 at codon 104 (CGC to TGC) (upper panel), resulting in substitution of an arginine with cysteine (R104C). This heterozygous missense mutation was also detected in one allele of the V2R gene of the second daughter (middle panel) and the mother (lower panel).

are anorexia or vomiting (70%), growth disturbance (50%), fever (40%), constipation (33%), polydipsia (13%) and mental retardation (3%) [11]. The patient had no such symptoms. His history indicated the possibility of an inherited X-linked recessive disorder. This suggested that his NDI may have been due to a compromised V2R, because most patients with AQP2-dependent NDI have autosomal recessive or autosomal dominant NDI, which is not carried on a sex chromosome. Therefore, males and females are equally likely to inherit it [1]. However, since the patient's second daughter had a mild degree of polyuria and polydipsia, it was difficult to distinguish between the two types of disorders, V2R-dependent NDI and AQP2-dependent NDI, based only on such clinical examinations.

Recently, it has been reported that patients with AQP2-dependent NDI will show responses to DDAVP taking place outside the kidney, while patients whose NDI is dependent on V2R genes will not. The informative parameters are heart rate, diastolic blood pressure and facial flushing as clinical manifestations, and cAMP in blood and urine as a laboratory finding (Table 1). Initially, inherited NDI was divided into two types based on differences in urinary excretion of cAMP after administration of pitressin or DDAVP [2]. Later, it was demonstrated that after infusion of DDAVP, blood levels of cAMP increase in patients with acquired NDI caused by a drug such as lithium [12] or AQP2-dependent NDI, but do not change in patients with V2R-dependent NDI [5]. Recently, it was reported that urinary aquaporin excretion can be detected in patients with V2R-dependent NDI, but

neither in patients with AQP2-dependent NDI [13] nor in patients with acquired NDI [14]. However, measurement of cAMP or aquaporin 2 is not practical in the routine setting. The factors which can be easily and accurately measured are tPA, factor VIII and vWF (Table 1). Many reports confirmed that responses of tPA, factor VIII and vWF in blood to DDAVP infusion are normal in NDI caused by an AQP2 mutation and acquired NDI [14] as well as central diabetes insipidus as healthy subjects [16], while there is no response to DDAVP infusion in NDI caused by V2R mutations (Table 1) [1–8]. The patient described here had no medication which caused the acquired NDI and showed no response of factor VIII and vWF blood levels to DDAVP infusion. Accordingly, these results suggested that he had inherited NDI caused by V2R anomaly.

Indeed, molecular genetic analysis demonstrated that the patient had the same V2R missense mutation as reported by Inaba *et al.* [9]. This mutation involves a substitution of cysteine for arginine at position 104 (R104C), which is located in the first extracellular loop of the V2R. Inaba *et al.* [9] reported that the R104C mutation reduces ligand-binding capacity and adenylyl cyclase activity, and that the sulfhydryl group of the cysteine at residue 104 of the mutant receptor is primary responsible for the dysfunction. Furthermore, the patient's mother and his second daughter aged 13 years were heterozygous for this mutation. This family with an R104C mutation represents the second reported such occurrence of this type. The first report by Inaba *et al.* [9] in 2001 was also in a Japanese family.

Table 1. Clinical and laboratory findings on the response to 1-deamino-8D-arginine vasopressin infusion either common to or distinguishing among vasopressin 2 receptor (V2R) dependent-nephrogenic diabetes insipidus (NDI), aquaporin 2 (AQP2)-dependent NDI and acquired NDI in this case.

	V2R-NDI	AQP2-NDI	Acquired NDI	This case
Facial flushing	absent	present	present	absent
Heart rate	→	↑	↑	→
Diastolic blood pressure	→	↓	↓	→
cAMP				
Blood	→	↑	↑	?
Urine	→	↑	↑	?
Urinary aquaporin 2	→	↓	↓	?
Factor VIII	→	↑	↑	→
von Willebrand factor	→	↑	↑	→
Tissue-type plasminogen activator antigen	→	↑	?	?

→: unchanging, ↓: decrease, ↑: increased, ?: unknown

Their patient was also diagnosed at a similar age of 53 years as this case, and the two satellite families are now living at the near places located at Niigata and Fukui prefectures which faced Sea of Japan, respectively, where the distance between the two satellites was about 300 km. Accordingly, the two patients may share common ancestors.

By 2002, 179 mutations of the gene have been recorded worldwide since the first case was reported in 1992 [17]. The inherited NDI in most patients is discovered in the period from infancy to adolescence [17]. These findings suggest that compared with other forms of hereditary V2R-dependent NDI, that caused by the R104C mutation may not trigger a serious acute outcome but rather slowly progressive disease [17].

Although he had no awareness of being sick, our patient had bilateral non-obstructive hydronephrosis. A review of the literature indicated a strong association between hereditary NDI and non-obstructive hydronephrosis [18]. Accordingly, it is advisable to seek to diagnose and treat inherited NDI as early as possible, even though a clinically-apparent outcome is long-delayed.

In conclusion, we report a case of mutant vasopressin receptor (R104C)-dependent nephrogenic diabetes insipidus with bilateral non-obstructive hydronephrosis distinguished from aquaporin-dependent nephrogenic diabetes insipidus by the responses of factor VIII and von Willebrand factor to 1-deamino-8-D-arginine vasopressin in a middle aged man.

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