Novel Mutant Vasopressin-neurophysin II Gene Associated with Familial Neurohypophyseal Diabetes Insipidus

MASASHI MIYAKOSHI, KYUZI KAMOI, TAKASHI MURASE*, YOSHIHISA SUGIMURA* AND YUTAKA OISO**

Department of Internal Medicine, Division of Endocrine and Metabolism, Nagaoka Red Cross Hospital, Nagaoka, Niigata 940–2085, Japan
*Department of Teratology and Genetics, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Aichi 464–8601, Japan
**Department of Endocrinology and Diabetology, Nagoya University, School of Medicine, Nagoya, Aichi 466–8550, Japan

Abstract. We describe a novel missense mutant of arginine vasopressin (AVP)-dependent neurohypophyseal diabetes insipidus in an autosomal dominant family. A 54-year-old woman was admitted to our hospital because of thyroidectomy for thyroid cancer. After thyroidectomy she was found to have hypernatremia and polyuria and polydipsia both of which had been present from childhood. She had no obstructive hydronephrosis. Her father, father’s younger sister and her third son also had polyuria and polydipsia. Basal plasma AVP concentration at normal plasma osmolality was normal but did not respond to increased plasma osmolality despite hyperosmolality during infusion of hypertonic saline infusion, indicating that plasma AVP secretion was impaired. Sodium concentration in urine and urine osmolality were low and increased after nasal administration of DDAVP. There was a diminished but bright signal of pituitary posterior gland on magnetic resonance T1 weighted image. Molecular genetic analysis demonstrated that the patient and her son had a single heterozygous missense mutation (G\textsuperscript{174}A) at nucleotide 1829 in 1 AVP allele, yielding an abnormal AVP precursor with lacking Glu-47 in its neurophysin II moiety. The abnormal AVP precursor may be related to the impaired AVP secretion.

Key words: Familial neurohypophyseal diabetes insipidus, AVP secretion, MRI of posterior gland, AVP gene

NEUROHYPOPHYSEAL diabetes insipidus (NDI) is caused by a deficiency of arginine vasopressin (AVP). Familial NDI (FNDI) is a hereditary form of diabetes insipidus [1], which is caused by diverse mutations in one allele of the gene that encodes the AVP precursor protein and AVP-neurophysin (NP) II [2]. Since the mutation in this gene was first reported in 1991 by Ito et al. [3], there have been 46 different mutations identified to date [2, 4–8]. Although the majority of mutations are located in the coding sequence of the signal peptide or the NPII domain [2], 18 different allelic variants are reported [2]. Of them, Yuasa et al. [9] reported a 3-bp deletion (AGG) out of 2 consecutive AGG sequences (nucleotides 1824–1829) in 1 AVP allele, which yields an abnormal AVP precursor lacking Glu-47 in its NP II moiety. Since Glu-47 is essential for NP molecules to form a salt bridge with AVP [10], the lacking Glu-47 would impair the function of NP as a carrier protein AVP. As a result, AVP probably undergoes accelerated proteolytic degradation [2, 9–11].

Here, we report a single heterozygous missense mutation (G→A) at nucleotide 1829 in 1 AVP allele in patients with FNDI, yielding an abnormal AVP precursor lacking Glu-47 in its NP II moiety as reported by Yuasa et al. [9].

Case Report and Methods

A 54-year-old woman was admitted to our hospital because of thyroidectomy for thyroid cancer. After the thyroidectomy, the patient was found to have hyper-
natremia (158 mEq/l) with polyuria and polydipsia. Since she had polyuria and polydipsia from when she was 12 years old, she had no awareness or symptoms of disease until admission. She received hysterectomy for myoma uteri at the age of 40 years in Yukiguni-Yamato General Hospital. At the time, she was diagnosed as primary polydipsia with bilateral non-obstructive hydronephrosis as shown by drip infusion pyerography, since she had normal level of plasma AVP concentration (0.6 pg/ml) as measured by the RIA method previously reported [12–13] although she had polyuria (5000–7000 ml/day) and polydipsia (5000–7000 ml/day). Before admission, she received Ca$^{2+}$ antagonist for hypertension. Also, her father and father’s younger sister and her third son (age 22 years) had polyuria and polydipsia (Fig. 1).

On admission, height was measured as 156 cm and weight 66.4 kg. Blood pressure was 164/110 mmHg, and pulse rate was regular at 72/min. Body temperature was 36.6°C. Her tongue was dry.

General laboratory data on examination day showed that concentrations of sodium, urea nitrogen and creatinine in blood and plasma osmolality were 139 mEq/l, 11.0 mg/dl, 0.7 mg/dl and 282 mosmol/kg, respectively, while sodium concentration in urine and urine osmolality were 14 mEq/l and 73 mosmol/kg, respectively. Urinary volume was 6000 to 8000 ml/day and intake-volume 4000 to 7000 ml/day. Concentrations of fasting plasma glucose (81 mg/dl), serum free triiodothyronine (2.14 pg/ml) and serum free thyroxine (1.09 ng/dl) were within normal range. Abdominal echogram showed no evidence of obstructive hydronephrosis.

Basal plasma osmolality and AVP concentration were 292 mosmol/kg and 0.98 pg/ml, respectively. Plasma AVP concentration at 308 mosmol/kg of plasma osmolality after infusion of 5% hypertonic saline was 1.18 pg/ml using the same RIA method described above [12–13], indicating that the pattern of plasma AVP secretion was flat, although its secretion was impaired (Fig. 2). Urinary volume decreased from 6.7 to 3.3 ml/min, urinary osmolality increased from 133 to 375 mosmol/kg and free water clearance decreased from 8.77 to –0.74 ml/min 4 hours after nasal administration of 10 μg of DDAVP, while plasma AVP concentration decreased from 0.81 to <0.2 pg/ml at the same time.

Fig. 1. Pedigree of familial neurohypophyseal diabetes insipidus relatives in this case. Circles, female; squares, men. Roman numerals, the generation. Open square and open circle with a central black dot, and solid circle and solid square, subjects with polyuria and polydipsia. Solid circle and solid square, subject for the mutation. A slash (/) through a symbol indicates that the subject is deceased. The arrow indicates the index cases.
Basal blood concentrations of TSH (3.92 μIU/ml), LH (20.8 mIU/ml), FSH (55.0 mIU/ml), PRL (4.3 ng/ml), GH (0.1 ng/ml) and ACTH (36.8 pg/ml) were within normal range, which normally increased after injections of TRH (500 μg), LH-RH (100 μg), GRH (100 μg) and CRH (100 μg).

There was a diminished but bright signal of pituitary posterior gland on magnetic resonance T1 weighted image (T1WI-MRI) (Fig. 3).

In her third son, plasma sodium concentration and osmolality on ad libitum water and sodium intake were 144 mEq/l and 290 mosmol/kg, respectively, while urinary sodium concentration and osmolality were 46 mEq/l and 207 mosmol/kg, respectively. Plasma AVP concentration was less than 0.2 pg/ml. The changing levels of plasma AVP after the stimulation by the infusion of hypertonic saline were not measured.

Mutation analysis of the AVP gene was performed. Informed consent for DNA analysis was obtained from the patient and her third son, and was approved by the institutional review board.

Genomic DNA was extracted from peripheral white blood cells using standard procedures. The entire AVP gene was amplified by PCR analysis using six sets of oligonucleotide primers as described previously [3, 9]. Direct sequencing of the PCR fragment was performed by the dideoxy procedure using a sequencing system (BigDye Terminator v3.1 Cycle Sequencing Kit; Applied Biosystems, Foster City, CA, USA) and an automatic DNA sequencer (ABI 3100-Avant Genetic...

![Fig. 2. Relationship between plasma concentration of AVP and plasma osmolality. The open circles connected by solid line represent the relation during infusion of 5% saline for 2 hours in this case, while the solid circles show the relation in normal subjects. Usually, plasma AVP levels in patients with neurohypophyseal diabetes insipidus (NDI) are less than 0.50 pg/ml at above 300 mosmol/kg of plasma osmolality. No. 1 connecting solid line represents subnormal but osmoregulated AVP secretion (Type 1), No. 2 connecting solid line osmoreceptor defect (Type 2), No. 3 connecting solid line partial NDI (Type 3) and No. 4 connecting solid line complete NDI (Type 4).](image)

![Fig. 3. Sagittal image. There was a diminished but bright signal of pituitary posterior gland on magnetic resonance T1 weighted image in this case.](image)

![Fig. 4. Section of the sequencing chromatograms obtained by dye terminator sequencing of PCR amplified exon 2 of the AVP-NPII gene in the patient and her son. The arrow shows a heterozygous mutation from G to A.](image)
As a result, the patient and her son were shown to have a single heterozygous missense mutation (G→A) at nucleotide 1829 in 1 AVP allele (Fig. 4). This mutation involves a substitution of lysine for glutamic acid at position 47, creating a lack of Glu-47 which is essential for forming a salt bridge between AVP and NP-II [10].

**Discussion**

This case is FNDI, because the patient and her son as well as her father and father’s younger sister had polyuria and polydipsia from childhood [1]. Most patients with FNDI have a deficiency of plasma AVP secretion such that the plasma AVP level is low and does not respond to increased plasma osmolality [14–16]. However, our patient had a normal level of plasma AVP at normal plasma osmolality. This view is supported by the finding that the bright signal of pituitary posterior gland on T1WI-MRI was diminished but present in this case. Generally, patients with NDI caused by a complete deficiency of plasma AVP secretion have no bright signal of pituitary posterior gland on T1WI-MRI [17]. However, there is a presence of the bright spot of pituitary posterior gland on T1WI-MRI in some patients with FNDI [18–20]. Two patients with FNDI associated with abnormal AVP precursor lacking Glu-47 as in this case in its NP-II moiety due to a 3-bp deletion (AGG) out of two consecutive AGG sequences (nucleotides 1824–1829) were reported by Yuasa et al. [9] as having a bright signal of pituitary posterior gland on T1WI-MRI, while three patients in the same family had no bright signal of it [18]. Interestingly, the former patients were found to have NDI at adult age and the latter patients at early childhood. In this case, NDI developed progressively with time. Therefore, a presence of the bright spot of pituitary posterior gland on T1WI-MRI in patients with FNDI may be related to the duration and degree of development of the NDI.

In this patient, a large amount of urinary volume was excreted although plasma AVP concentration was normal as if was diagnosed as primary polydipsia before. This may be explained by the decreased sensitivity of plasma AVP to renal tubules due to a long-term deficiency of AVP secretion. In this case, the increase in urine osmolality and the decrease in free water clearance 4 hours after nasal administration of DDAVP was half as compared with those using the same method as this case in patients with NDI reported by Katayama et al. [21], indicating that the response of plasma AVP to renal tubules was diminished.

The normal level of plasma AVP concentration did not respond to increased plasma osmolality produced by the infusion of 5% hypertonic saline. Usually, in NDI patients the plasma AVP levels measured by the RIA method reported here are less than 0.50 pg/ml on ad libitum intake of water, and they remain low when plasma osmolality is elevated above 300 mosmol/kg by hypertonic saline infusion [12]. Therefore, this case shows a secretion of plasma AVP that is impaired but its pattern is a persistent secretion of plasma AVP with no major fluctuation in plasma AVP concentration despite marked hyperosmolality. Using this method of 5% hypotonic saline infusion, four patterns of AVP response for plasma osmolality (Fig. 2) were reported in NDI patients [22]. Type 1 is subnormal but osmoregulated AVP secretion where the incremental increase in plasma AVP concentration for each unit rise is reduced. Type 2 is osmoreceptor defect such that the pattern of AVP response is characterized by persistent secretion of AVP with no major fluctuation in plasma AVP concentration despite marked hyperosmolality. Type 3 is partial NDI where plasma AVP levels are low or undetectable but rise slightly and erratically with hypertonicity. Type 4 is complete NDI whose plasma AVP levels are completely undetectable. In this case, the pattern resembled type 2. The similar levels of plasma AVP (1.00–1.80 pg/ml) measured by the same method of RIA as this case are observed in patients with FNDI associated with abnormal AVP precursor lacking Glu-47 in its NP-II moiety as reported by Yuasa et al. [9] and Miyamoto et al. [18].

In this case, molecular analysis demonstrated that the patient and her son had a single missense mutation (G→A) at nucleotide 1829 in 1 AVP allele. This mutation involves a substitution of lysine for glutamic acid at position 47, resulting in a lack of Glu-47 which is essential for forming a salt bridge between AVP and NP-II [10]. This family with a single missense mutation at nucleotide 1829 represents the first reported occurrence of this type. The lack of Glu-47 was first reported by Yuasa et al. [9] and then two families by Rittig et al. [14] who reported abnormal NP molecule lacking AVP-binding affinity. The lack of AVP-binding affinity may accelerate proteolytic degradation of AVP, producing a deficiency of AVP secretion [11].
absence of a bright spot in the pituitary posterior gland on T1WI-MRI in some patients with FNDI may be explained by the presence of stored AVP in the cytoplasm associated with the abnormal processing of AVP synthesis. Miyamoto et al. [18] and Miller [23] speculated that the impaired AVP-NP II tetramerization resulted from abnormal precursor molecules within the hypothalamic cells could lead to a progressively retrograde and toxic degeneration of the AVP NP-II neurons, which could be responsible for the disappearance of the posterior pituitary signal. Indeed, the lack of Glu-47 enhances dimerization or tetramerization of the neurophysin molecule and accumulates large amounts of mutant prohormone in the endoplasmic reticulum with the resulting that stimulated secretion of stored AVP is impaired as in the state of osmoreceptor defect of type 2 [11]. In this case, administration of DDAVP suppressed plasma AVP concentration and its response to urinary concentration diminished in comparison with that in other patients with NDI [21], indicating functions of secretion and activity of AVP may be normally reserved.

In conclusion, in the patient with a lack of Glu-47, NDI may be due to impaired plasma AVP secretion caused by slowly degenerated AVP neurons and/or disturbance of transport mechanism of AVP prohormone to AVP rather than due to an impaired activity of abnormal mutant AVP, which is produced by a diminished export of AVP prohormone from endoplasmic reticulum to the trans-Golgi network [11]. The relation between the persistent secretions of plasma AVP with no major fluctuation in plasma AVP concentration despite marked hyperosmolality may be explained by an abnormal processing mechanism in AVP synthesis. Further study is needed to clarify it.

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References


