A case of central diabetes insipidus due to neurophysin II gene abnormality diagnosed based on a family history of nocturnal enuresis

Lucia Sugawara1,2, Takaaki Nakamura1, Yoshitaka Ishizuka1 and Hiroshi Maegawa2

1) Department of Endocrinology and Metabolism, Omihachiman Community Medical Center, Omihachiman, Shiga 523-0082, Japan
2) Department of Medicine, Shiga University of Medical Science, Otsu, Shiga 520-2092, Japan

Abstract. The etiology of central diabetes insipidus (DI) is classified into (1) idiopathic, (2) familial, and (3) secondary. Of these, familial central diabetes insipidus shows an autosomal dominant inheritance. We herein report a case in which this disease was diagnosed based on a family history of nocturnal enuresis. A 40-year-old man had had symptoms of polydipsia, polyuria and nocturia since childhood and found that his daughter had the same symptoms. Despite reaching nine years old, his daughter’s nocturnal enuresis still had not improved, resulting in her consulting a pediatrician. She was suspected of having familial neurohypophyseal diabetes insipidus (FNDI) based on her family history and was referred along with her father for a detailed examination and treatment. A hypertonic saline load test (HSLT) to evaluate the arginine vasopressin (AVP) reaction was performed in both the proband and his daughter. The results showed no increase in AVP levels in response to high plasma osmolality. The water deprivation test (WDT) revealed he was suffering from partial DI. Based on the above findings and considering the possibility of familial central diabetes insipidus, we performed a gene mutation analysis of AVP-neurophysin II (NPII). Both the father and daughter had an exon 2 abnormality in this gene (c232_234delGAG; p.Glu78del), and this gene mutation is known to cause NPII protein abnormality, abolishing the function of AVP as a carrier protein. This case was considered to have provided an opportunity to understand the role of an NPII gene abnormality in familial central diabetes insipidus.

Key words: Familial neurohypophyseal diabetes insipidus, Arginine vasopressin, Neurophysin II, Polydipsia

AVP is an antidiuretic hormone (ADH) and a peptide hormone synthesized by specialized neurons in both the supraoptic and paraventricular nuclei of the hypothalamus [1]. Genetically, different stimuli for ADH secretion primarily affect gene transcription and secondarily affect posttranscriptional regulation in these neurons [2]. ADH is transported from the bodies of these nuclei to the posterior pituitary along with neurophysin II (NPII).

Diabetes insipidus (DI) is a disorder involving a large volume of dilute and hypotonic urine excretions, and we have observed many causes for this disease; however, familial neurohypophyseal (FN) DI is a rare inherited disorder with an autosomal dominant inheritance pattern. It is characterized by persistent polydipsia and polyuria induced by deficient or absent secretion of ADH [3, 4].

Genetically, the 2.5-kb ADH-NPII gene consists of 3 exons. These proteins are synthesized as part of ADH precursor molecule by way of a genetic transcription in the short arm of chromosome 20 (20p13) [5]. The distribution of the structure of NPII can have two main effects on ADH action and metabolism [6, 7]. First, there may be a decline in the number of binding sites and activity of endopeptidase responsible for the cleavage of ADH [8]. Second, there may be a change in the pattern of polymerization of NPII and its binding of ADH, which in turn may result in a specific enzymatic degradation of the hormone [9, 10]. Both of these effects can lead to deficiency in the amount of available ADH.

Among the ADH-NPII mutations related to FNDI, deletion mutations have been reported in only a few cases [11, 12]. We herein report a Japanese family with FNDI, associated with the heterozygous deletion of three nucleotides in exon 2 (c.232_234delGAG; p.Glu78del) of the ADH-NPII gene.
Case Report

The proband was a 40-year-old male admitted to our hospital due to complaints of thirst, polydipsia, nocturia and polyuria; he reported having had the same symptoms of polydipsia with a history of nocturnal enuresis since elementary school, and that his daughter had these symptoms as well. In detail, he usually drank about 2 liters of water with each meal and went to the bathroom more than 10 times a day. At the same time, his daughter, nine years old, underwent a pediatric examination for nocturnal enuresis and was diagnosed with potential partial central DI. The proband’s symptoms of polydipsia, polyuria and nocturia were so severe that we decided to start the administration of desmopressin acetate hydrate formulation of 180 μg a day before the tests for diagnosis of DI were performed. This daily dose of this formulation was considered sufficient to release his clinical symptoms.

Firstly, we checked the pedigree of the family (Fig. 1). Although the proband’s mother had never been tested for plasma ADH levels or undergone a genetic analysis, she had had the same symptom of persistent polydipsia and polyuria, suggesting that she might have the same disease. This familial pedigree suggested autosomal dominant transmission. Furthermore, we performed HSLT, (5%, 0.05 mL/kg/min for 2 h) to assess the vasopressin reaction in the proband on the day after admission, and the results showed no increase in the ADH levels in response to high plasma osmolality (Fig. 2). Consequently, the basal levels of serum of ADH concentrations were little different between the proband and his daughter with possessing genetically the same AVP: 4.3 pg/mL in the proband, and 2.1 pg/mL in the daughter. Since we checked those values with Yamasa’s anti-ADH antibody which has a cross-reactivity of 0.001% or less with desmopressin [13], there is no possibility that it has detected desmopressin acetate taken orally. Thus, the most important findings in HSLT were thought to be the fact that neither the proband nor the daughter has an increase in ADH in proportion to the osmolality increase.

Next, to determine whether or not the patient’s nocturia and polyuria were due to real disease, we performed WDT on the next day after admission, including the determination of both the urine osmolality and the concentrations of ADH. The WDT is widely used for the differential diagnosis of polyuria-polydipsia syndrome. However, it is inconvenient and may not always be precise in differentiating the partial forms of DI from primary polydipsia. It was recently reported that the direct measurement of hypertonic saline-stimulated plasma copeptin had greater diagnostic accuracy than the WDT in patients with hypotonic polyuria [14]. Therefore, we attempted to assess the plasma copeptin concentration of the proband, but unfortunately, there was no commercially available method of checking this concentration at the time. For this reason, we performed the WDT via the following procedure: Fluid intake was stopped at 8:00 starting the test, and this test was prolonged for more than 6 h until reaching the 2.5%–3% decrease in the weight [15]. We first confirmed whether or not the proband indeed had polyuria. For the WDT, the data of every voided urine sample were recorded, and the urine osmolality was measured (Fig. 3B, C). We also measured

![Fig. 1 Pedigree of the familial neurohypophyseal diabetes insipidus relatives in this case. Circle, female; square, male. Roman numeral, generation. Open circle with a central black dot, obligate carrier who does not manifest the disease with polyuria or polydipsia. Solid circle and solid square, subject with the mutation. The arrow indicates the index case.](image)

![Fig. 2 Relationship between the plasma concentrations of ADH and plasma osmolality. The solid squares and circles connected to solid lines represent the relationship during 5% saline administration for 2 h in this proband and his daughter, respectively, while the solid areas indicate the relationship in control subjects.](image)
the serum concentrations of ADH at 0, 3 and 6 h after starting the test. The patient received an intravenous injection of 5 units of Pitressin at the endpoint of this test (Fig. 3A). The data revealed he was suffering from partial DI.

Finally, after obtaining informed consent from both the proband and his daughter, blood samples were collected from them to confirm whether or not genetic abnormalities were present. Genetic DNA was isolated from peripheral blood leukocytes using a QuickGene DNA whole blood kit S (KURABO Industries, Okayama, Japan) following the manufacturer’s instructions. All three exons of the ADH-NPII genes were amplified by polymerase chain reaction (PCR) with the primers as described previously [16]. Sanger sequencing was performed on the PCR products using an ABI 3730 sequencer, as described previously [17]. The reference sequence and exon numbering are according to the Genbank accession number NM_000490, with the A of the ATG start codon at position 1. The pathogenic heterozygous deletion of three nucleotides from AVP: c. 232_234 del GAG was identified in exon 2 of the ADH gene (Fig. 4). This deletion is predicted to result in the deletion of glutamic acid at position 78 (AVP: p. Glu78del). The plasma levels of PRL, GH, IGF-1, FSH, LH, ACTH, cortisol, TSH, free T4, and free T3 were all within the normal ranges. T1-enhanced magnetic resonance imaging of the pituitary showed a normal shape of the neurohypophysis and pituitary stalk but loss of posterior pituitary hyperintensity.

**Discussion**

The presence of polyuria is thought to be consistent with the results of both HSLT, and WDT suggesting that partial central DI might have occurred. However, the basal serum concentrations of ADH during the HSLT and WDT seemed to be somewhat variable. In the case of DI, we should always consider which anti-AVP antibody is being used in assay, as the test results vary depending on which antibody is applied. And we confirmed that Yamasa’s ant-AVP antibody was used for all of these assays, indicating that there was no measurement variability. Thus, ADH concentration in the pro-
band was measured as his real value whether he took desmopressin acetate hydrate formulation before these tests. These facts indicated that even though the proband with FNDI with ADH-NPII partial genetic disorder could not secrete sufficient ADH in response to the osmolality, he might secrete more than 4.0 pg/mL of basal ADH to the circulation.

Actually, when the already high serum Na concentration increased following hypertonic saline infusion, no responding increase in ADH secretion was found. In addition, the renal tubular function in response to Pitressin revealed to be normal because of increased urine osmolality. These results suggest that the synthesis and secretion of normal ADH may be impaired. Given the above findings, we searched for an abnormality in the ADH gene. Consequently, we diagnosed the patient and his daughter with familial central DI due to a gene mutation in the region encoding NPII. Glutamic acid by a GAG deletion on exon 2 of the ADH-NPⅡ gene by the direct sequence method and an in-frame deletion of (c.232_234delGAG; p.Glu78del) were identified.

Most of the variants of this gene are situated in the NPII gene, with seven variants detected [9]. As has been described, seven intrachain disulfide bonds are formed in NPII, which may be essential for stabilizing the protein. In addition, a case of FNDI with the same genetic abnormality as the present case was described in South Korea in 2008 [18]. While the deletion of Glu78 was followed by a frameshift that affected only those three codons, with the remaining exon 2 codons entirely preserved in the Korean family, the remaining codon sequences of the downstream alleles in the present case were completely different from normal, suggesting that these two families are totally independent. However, the pathological mechanisms are expected to be similar in both cases.

A previous report suggested that Glu78 is essential for allowing NPII molecules to form salt bridge disulfide bonds, the function of NPII as a carrier protein would be impaired. Therefore, most of the ADH in our proband may undergo accelerated proteolytic degradation [10]. In addition, another possibility of this inability of this ADH mutation is thought to exhibit dominant negative characteristics, allowing polyuria symptoms to manifest despite the presence of normal amounts of ADH [19]. Furthermore, in cell and animal experiments, the accumulation of abnormal proteins in the endoplasmic reticulum reportedly induces apoptosis of nerve cells [20]. These results indicate that symptoms such as polydipsia and polyuria might have gradually progressed over the first several years of life, with complete ADH secretion deficiency not being noticed at birth. However, the results of these basic studies have not seemed to match the case of FNDI we experienced this time. The reason was, first of all, the fact that the secretion of basal ADH in the proband was higher than we expected. The proband’s ADH was higher than the daughter’s basal value suggests that ADH-producing cells had been surviving but not apoptotic. In addition, it suggested that ADH was transferred to the posterior pituitary gland (PPG) by some mechanisms or ADH producing cells might directly secrete this ADH.
hormone to the circulation even if there was a structural abnormality of NPII. At least, this transfer neither appeared to be supplied as needed nor ADH was regularly stored in the PPG because MRI did not show hyperintensity in PPG and both HSLT and WDT revealed this. Further research will be needed to confirm this.

The present proband has been treated with desmopressin oral formulation containing 180 µg a day, which has also been developed as a sublingual lyophilisate (melt) formulation. Since his symptoms of polydipsia, polyuria and nocturia were severe, we decided to start the administration of this formulation before the diagnosis of DI was made. That did not cause any confusion concerning with the tests results at all.

This sublingual formulation improves the bioavailability of desmopressin by approximately 60% compared to the desmopressin tablet itself [21]. The goal of chronic management in this patient should be to provide complete, around-the-clock control of his polyuria with minimal risk of hyponatremia due to excessive water retention. This can usually be achieved by administering desmopressin-melt formulations of antidiuretic therapy and educating the patient on the importance of strictly limiting fluid intake to the amounts required to satisfy thirst. As he has shown no issues with his serum Na concentrations since administration of this medication was started, this is thought to be a very effective therapy for FNDI.

**Disclosure**

1. The authors declare no conflicts of interest.
2. Institutional review board statement

This study was approved by the Institutional Review Board (IRB) of Omihachiman Medical Community Center, Japan. Both the proband and his daughter provided informed consent forms according to the institutional guidelines, and the genetic study conformed to the IRB protocols.

**References**

1. Brownstein MJ, Russell JT, Gainer H (1980) Synthesis, transport, and release of posterior pituitary hormones. Science 207: 373–378.
2. Ito M, Mori Y, Oiso Y, Saito H (1991) A single base substitution in the coding region for neurophysin II associated with familial central diabetes insipidus. J Clin Invest 87: 725–728.
3. Blackett PR, Seif SM, Altmiller DH, Robinson AG (1983) Familial central diabetes insipidus: vasopressin and nicotine stimulated neurophysin deficiency with subnormal oxytocin and estrogen stimulated neurophysin. Am J Med Sci 286: 42–46.
4. McLeod JD, Kovacs L, Gaskill MB, Rittig S, Bradley GS, et al. (1993) Familial neurohypophyseal diabetes insipidus associated with a signal peptide mutation. J Clin Endocrinol Metab 77: 599A–599G.
5. Bahnson U, Oosting P, Swaab DF, Nahke P, Richter D, et al. (1992) A missense mutation in the vasopressin-neurophysin precursor gene cosegregates with human autosomal dominant neurohypophyseal diabetes insipidus. EMBO J 11: 19–23.
6. Di Iorgi N, Napoli F, Allegri AE, Olivieri I, Bertelli E, et al. (2012) Diabetes insipidus diagnosis and management. Horm Res Paediatr 77: 69–84.
7. Fenske W, Alloio B (2012) Current state and future perspectives in the diagnosis of diabetes insipidus: a clinical review. J Clin Endocrinol Metab 97: 3426–3437.
8. Yuasa H, Ito M, Nagasaki H, Oiso Y, Miyamoto S, et al. (1993) Glu-47, which forms a salt bridge between neurophysin-II and arginine vasopressin, is deleted in patients with familial central diabetes insipidus. J Clin Endocrinol Metab 77: 600–604.
9. Garcia-Castaño A, Madariaga L, Pérez de Nanclares G, Vela A, Rica I, et al. (2020) Forty-one individuals with mutations in the AVP-NPII gene associated with familial neurohypophyseal Diabetes Insipidus. J Clin Endocrinol Metab 105: 1112–1118.
10. Calvo B, Bilbao JR, Rodríguez A, Rodríguez-Arnao MD, Castaño L (1999) Molecular analysis in familial neurohypophyseal diabetes insipidus: early diagnosis of an asymptomatic carrier. J Clin Endocrinol Metab 84: 3351–3354.
11. Rittig S, Robertson GL, Siggaard C, Kovács L, Gregersen N, et al. (1996) Identification of 13 new mutations in the vasopressin-neurophysin II gene in 17 kindreds with familial autosomal dominant neurohypophyseal diabetes insipidus. Am J Hum Genet 58: 107–117.
12. Toustrup LB, Kvistgaard H, Palmfeldt J, Bjerre CK, Gregersen N, et al. (2018) The novel Ser18 del AVP variant causes inherited neurohypophyseal diabetes insipidus by mechanisms shared with other signal peptide variants. Neuroendocrinology 106: 167–186.
13. Ito T, Takagi J (2017) The differential diagnosis of central diabetes insipidus by arginine-vasopressin measurement using high-sensitivity radioimmunoassay. J Aichi Med Univ Assoc 45: 33–40.
14. Fenske W, Refardt I, Chifu I, Schnyder I, Winzeler B, et al. (2018) A Copeptin-Based approach in the diagnosis of diabetes insipidus. N Engl J Med 379: 428–439.
15. Zerbe RL, Robertson GL (1981) A comparison of plasma vasopressin measurements with a standard indirect test in the differential diagnosis of polyuria. N Engl J Med 305: 1539–1546.
16. Ito M, Mori Y, Oiso Y, Saito H (1991) A single base substitution in the coding region for neurophysin II associated with familial central diabetes insipidus. *J Clin Invest* 46: 725–728.

17. Ueta Y, Taniguchi S, Yoshida A, Murakami I, Mitani Y, *et al.* (1996) A new type of familial central diabetes insipidus caused by a single base substitution in the neurophysin II coding region of the vasopressin gene. *J Clin Endocrinol Metab* 81: 1787–1790.

18. Lee YW, Lee KW, Ryu JW, Mok JO, Ki CS, *et al.* (2008) Mutation of Glu78 of the AVP-NPII gene impairs neurophysin as a carrier protein for arginine vasopressin in a family with neurohypophyseal diabetes insipidus. *Ann Clin Lab Sci* 38: 12–14.

19. Ito M, Yu RN, Jameson JL (1999) Mutant vasopressin precursors that cause autosomal dominant neurohypophyseal diabetes insipidus retain dimerization and impair the secretion of wild-type proteins. *J Biol Chem* 274: 9029–9037.

20. Phillips JA 3rd (2003) Dominant-negative diabetes insipidus and other endocrinopathies. *J Clin Invest* 112: 1641–1643.

21. Birk J, Friberg MA, Prescianotto-Baschong C, Spiess M, Rutishauser J (2009) Dominant pro-vasopressin mutants that cause diabetes insipidus form disulfide-linked fibrillar aggregates in the endoplasmic reticulum. *J Cell Sci* 122: 3994–4002.