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LETTER TO THE EDITOR

Clinical and molecular analysis of a Chinese family with autosomal dominant neurohypophyseal diabetes insipidus associated with a novel missense mutation in the vasopressin–neurophysin II gene

Yongfeng Luo · Binbin Wang · Yu Qiu · Chuan Zhang · Chengluo Jin · Yakun Zhao · Qingguo Zhu · Xu Ma

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Abstract The objective of this study is to identify the genetic defects in a Chinese family with autosomal dominant familial neurohypophyseal diabetes insipidus. Complete physical examination, fluid deprivation, and DDAVP tests were performed in three affected and three healthy members of the family. Genomic DNA was extracted from leukocytes of venous blood of these individuals for polymerase chain reaction amplification and direct sequencing of all three coding exons of arginine vasopressin–neurophysin II *(AVP–NPII)* gene. Seven members of this family were suspected to have symptomatic vasopressin-deficient diabetes insipidus. The water deprivation test in all the patients confirmed the diagnosis of vasopressin-deficient diabetes insipidus, with the pedigree demonstrating an autosomal dominant inheritance. Direct sequence analysis revealed a novel mutation (c.193T>A) and a synonymous mutation (c.192C>A) in the *AVP–NPII* gene. The missense mutation resulted in the substitution of cysteine by serine at a highly conserved codon 65 of exon 2 of the *AVP–NPII* gene in all affected individuals, but not in unaffected members. We concluded that a novel missense mutation in the *AVP–NPII* gene caused neurohypophyseal diabetes insipidus in this family, due to impaired neurophysin function as a carrier protein for AVP. The Cys65 is essential for NPII in the formation of a salt bridge with AVP. Presence of this mutation suggests that the portion of the neurophysin peptide encoded by this sequence is important for the normal expression of vasopressin.

Keywords Diabetes insipidus · Autosomal dominant · Mutation · Vasopressin · Neurophysin II

Introduction

Autosomal dominant familial neurohypophyseal diabetes insipidus (adFNDI) is a rare inherited disease characterized by polydipsia, polyuria, and dehydration caused by deficient secretion of the peptide hormone, arginine vasopressin (AVP) [1]. AVP and its corresponding carrier neurophysin II (NPII), are synthesized as a composite precursor by the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus. The precursor is packaged into neurosecretory granules and transported axonally in the stalk of the posterior pituitary, En route to the neurohypophysis, the precursor is processed into the active hormone. Prepro-vasopressin has 164 amino acids and is encoded by the 2.5 kb *prepro-AVP–NPII* gene located in chromosome region 20p13. The *AVP–NPII* gene consists of three exons and two introns. Exon 1 of the *AVP* gene encodes the signal peptide AVP and the NH_2-terminal region of NPII. Exon 2 encodes the central region of NPII, and exon 3 encodes the COOH–terminal region of NPII and the glycopeptide [2, 3].

Disease onset is typically in infancy or adolescence in the affected individuals, with symptoms worsening throughout adulthood. To date, there have been more than 50 different mutations in the *AVP–NPII* gene encoding the AVP preprohormone [4]. The mutations occur in different types and

Yongfeng Luo, Binbin Wang and Yu Qiu contributed equally to this study.

Y. Luo · Y. Qiu · C. Zhang · C. Jin · Y. Zhao · Q. Zhu (✉)
Department of Urology, The Second Affiliated Hospital of Harbin Medical University, Harbin, China
e-mail: hydzqg@163.com

B. Wang · X. Ma
Department of Genetics, National Research Institute for Family Planning, Beijing, China

are located at different sites in the coding region, and affect amino acid residues that are essential for proper folding and dimerization of the neurophysin II moiety of the AVP preprohormone [5, 6]. This study analyzes a Chinese family with adFNDI associated with mutations in the *AVP–NPII* gene. We also report a novel mutation (c.193T>A) and a synonymous mutation (c.192C>A) identified in the *AVP–NPII* gene. The novel missense mutation resulted in the substitution of cysteine by serine at a highly conserved codon 65 of exon 2 of the *AVP–NPII* gene in all the affected individuals.

Subjects and methods

Subjects

After obtaining written informed consent, we studied only six living members of the Chinese pedigree of Han family presenting with adFNDI, as other members of the pedigree refused to participate. II.3 and II.4 had a chronic history of polyuria and polydipsia with onset of symptoms including edema of lower extremity, at about the ages 38 and 40 years, respectively. Subsequently, they were diagnosed with backache at a local hospital. The findings from color Doppler sonography revealed hydronephrosis and enlarged urinary tract, for which they consulted with an urologist at our hospital initially. The family member II.9 showed milder signs, while the other living members including III.8, III.12, IV.18 were unaffected (Fig. 1).

Methods

Clinical data and sample collection

All participants underwent full physical examination and fluid deprivation test plus desmopressin (using DDAVP to terminate the fluid restriction period used for differentiating central from nephrogenic diabetes insipidus). Urine was measured for specific gravity. All the samples of urine and serum were analyzed at the second affiliated hospital of Harbin Medical University (Harbin, China). Samples of blood were shipped to Department of Genetics, National Research Institute for Family Planning for DNA analysis (Beijing, China).

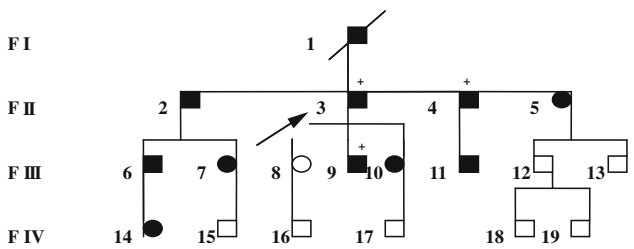

Fig. 1 Pedigree of the kindred. The individuals numbered are those who were available for mutation screening of the *AVP–NPII* gene. *Black* and *white* symbols represent clinically affected and unaffected individuals, respectively. Genetically tested individuals are indicated by the "+" symbol. The *arrow* to II.3 is propositus and indicates the carrier of the 1619T>A mutation

Molecular genetic procedures

Venous blood samples were obtained from six members of the pedigree and 100 normal controls. Genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kits (QIAGEN Science, Germantown, MD). All coding exons of *AVP–NPII* genes were amplified by polymerase chain reaction (PCR) with primers listed in Table 1. The PCR products were then sequenced from both directions with the ABI3730 Automated Sequence (PE Biosystems, Foster City, CA).The sequencing results were analyzed using Chromas (version 2.3) and compared with the reference sequences in the NCBI database.

Results

Clinical data

Analysis of the water deprivation test results was performed in the index patient, according to the standard procedures, along with the DDAVP test (Table 2). Patients voided the bladder before the administration and urinary flow was determined hourly. The water deprivation test demonstrated the urine concentration deficiencies in the affected individuals of the Chinese family: the urine specific gravity sharply increased in the affected individuals after DDAVP administration (AVP, 5 IU i.m.). The data supported the diagnosis of diabetes insipidus (DI), differentiating central from nephrogenic types [7]. However, in all the investigations, no abnormalities were seen in the three unaffected individuals of the family. Overall, the clinical findings were consistent with the diagnosis of central AVP-deficient DI. The patients were treated by DDAVP (II.3 and II.4: Minrin 0.1 mg × 4 t.i.d, p.o.; III.9: 0.1 mg × 2 t.i.d, p.o.). Although some family members refused to undergo the various tests details of family history obtained from the participants indicated seven living family members were suspected to have symptomatic vasopressin-deficient DI to varying degree. Although not tested, I.1 might be the first affected family member, and thus expected to have milder signs.

Molecular genetic analysis

Direct sequencing of *AVP–NPII* gene revealed a heterozygous T>A transition at position c.193, which resulted in the

Table 1 Primers used for DNA amplification by PCR

Exon	Forward (5′–3′)	Reverse (5′–3′)	Product length (bp)
AVP-II-1	AGCGCTGCAGTCACAGTAGA	GCTACCACCACCCATGACTT	521
AVP-II-2	CTCGCTGCGTTCCCCTCCAACCCCTCGACTC	CCCCCAGCCCCAGGCCCGCCCCCGCCGCGC	305
AVP-II-3	CCGTGCTCACACGTCCTC	CATTGGCGGAGGTTTATTGT	270

Primers for exons 1, 2, and 3: exon 3 and exon 2 have been previously reported [18]. Exon 1 has been slightly modified to improve sequencing results; cycle conditions include 96°C for 30 s, 65°C for 45 s, and 74°C for 30 s, 35 cycles

Table 2 Water deprivation and vasopressin tests in the index case

Experiment	Patients	Time	Blood pressure (mmHg)	Pulse	Weight (kg)	Volume (ml)	Urine temperature (°C)	Specific gravity	Corrected specific gravity
Water deprivation test	II.3	8:00	140/80	92	100				
		9:00	135/75	98	99.5	420	30	0.998	1.003
		10:00	135/75	75	99.2	380	27	0.996	1.001
		11:00	140/85	83	98.7	260	27.5	1.000	1.004
		12:00	130/90	87	98.3	310	28	0.998	1.002
		13:00	135/80	75	98.1	230	30	1.000	1.005
	II.4	10:15	146/100	88	75	620	32.5	0.996	1.002
		11:15	140/95	90	74.5	410	29.5	0.998	1.003
		12:15	140/95	85	74.3	247	33	0.998	1.004
		13:15	135/90	92	73.8	225	31	0.996	1.001
		14:15	135/90	90	73.7	230	30	1.000	1.005
	III.9	8:00	115/85	75	67.5	260	33	0.998	1.004
		9:00	110/80	80	67.2	250	29	0.996	1.001
		10:00	110/80	75	67	245	27.5	1.000	1.004
		11:00	125/85	81	67	300	28	0.998	1.002
		12:00	125/90	80	66.8	200	27.5	1.000	1.004
Vasopressin test	II.3	14:00	135/75	87	98.8	210	31	1.002	1.007
		15:00	140/85	75	98.6	100	30	1.005	1.010
		16:00	135/80	82	98.6	80	33	1.008	1.014
		17:00	135/75	90	98.7	50	29.5	1.012	1.017
		18:00	140/80	85	98.5	100	30	1.010	1.015
	II.4	15:15	130/90	88	74.3	270	28	1.006	1.010
		16:15	135/85	90	74.1	50	30	1.010	1.015
		17:15	135/85	88	74.1	40	29.5	1.010	1.015
		18:15	140/85	85	74	50	30	1.012	1.016
	III.9	13:00	115/85	88	67.3	200	33	1.005	1.011
		14:00	125/80	75	67.2	75	32	1.006	1.012
		15:00	120/80	85	67.2	50	32	1.009	1.015
		16:00	120/80	88	67	35	31	1.009	1.015

Intramuscular injection 5 IU of arginine vasopressin to terminate the water deprivation test

Corrected specific gravity = (urine temperature − 15)/3000 + specific gravity

substitution of cysteine by serine at the highly conserved codon 65 of exon 2, and a synonymous mutation c.192C>A at codon 64 of exon 2, changing GGC to GGA corresponding to Gly codon (Fig. 2). Meanwhile, the mutation was confirmed by restriction endonuclease analysis of PCR amplification products that contain the corresponding segment of the *AVP–NPII* gene. The mutation was a thymidine base substituted by adenine at nucleotide c.193(T>A) in exon 2, which altered codon 65 of the *prepro*-AVP–NPII sequence where the NPII moiety was derived from TGC to an AGC mutation. No other mutations were detected in exons 1, 2 or 3. The missense mutation and the synonymous mutation were found to be similar in all the affected individuals and co-segregated with the affected individuals in

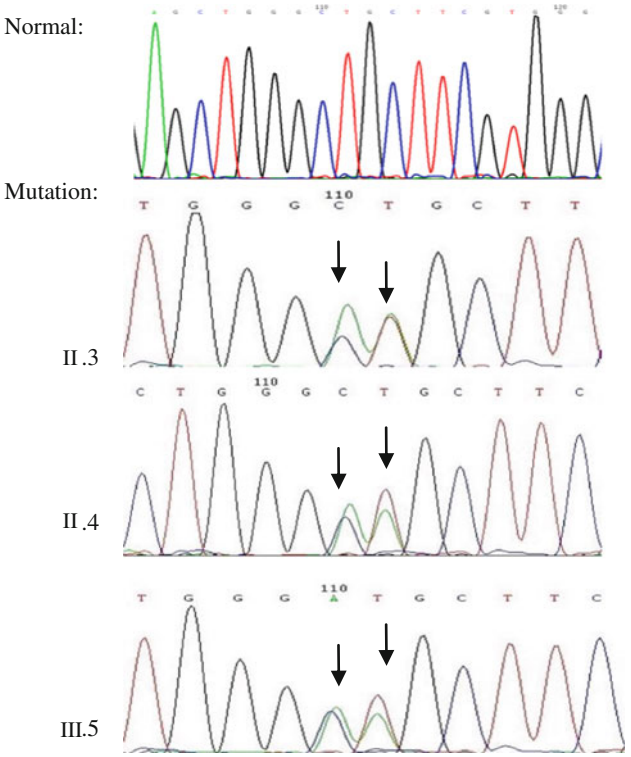

Fig. 2 Automated sequencing of the *AVP–NPII* gene**.** A portion of exon 2: the two superimposed chromatographic peaks demonstrate the heterozygous mutation c.193(T>A) and nonsense mutation c.192(C>A) found in the Chinese family. The *arrow* represents the novel mutation identified

the family but absent in the unaffected family members as confirmed by the sequencing of 100 control chromosomes.

Discussion

The mutations included intragenic deletions, missense and nonsense mutations affecting the signal peptide, the AVP moiety, or the AVP carrier protein (NPII). Over 50 distinct *AVP–NPII* gene mutations have been reported in adFNDI. However, only five nonsense mutations were reported, with almost a quarter affecting one of the seven disulfide bonds present in NPII that are important in determining its tertiary structure [5, 8, 9]. If only the mutations affecting the NPII moiety were considered, the proportion of the modified cysteine residues, forming disulfide bonds, rises to nearly 50%. Therefore, the mutations affecting the NPII play an important role in the adFNDI pathophysiology. The study demonstrated the clinical symptoms in this kindred, with a co-segregation of the novel mutation (c.193T>A) in the *AVP–NPII* gene. The heterozygous c.193T>A mutation identified in the neurophysin moiety corresponds with the different patterns of the other known mutations associated with initial mild symptoms worsening in severity with AVP deficiency. The novel mutation identified in the Chinese kindred resulting from the substitution of cysteine by serine at the highly conserved codon 65 of exon 2 of the *AVP–NPII* gene was seen in all the affected individuals. Only the affected individuals carrying a mutation showed the symptoms of the adFNDI. Therefore, c.193(T>A) is most likely a disease-causing mutation.

The pathogenesis of adFNDI is generally linked with deficient or insufficient function of mutant NPII molecules. NPII is responsible for safe axonal transport and prevention of intracellular AVP proteolysis. Mutant AVP prohormone is degraded by the cytosolic proteolytic system. The mutant precursor hormones are retained by the endoplasmic reticulum quality control mechanisms resulting in cytotoxic accumulation and protein aggregation in the neurons, or degeneration of magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus [2, 10–17]. The AVP peptide binds in a “pocket” formed by NPII, and thereby shielded from proteolytic degradation during the axonal transport of the secretory granule to the neurohypophysis where AVP is released, as needed [2, 18]. Therefore, heterozygous T>A transition at highly conserved codon 65 of exon 2, resulting in the substitution of cysteine by serine could disorder the NPII protein. The mutant NPII with a deficient or insufficient function interferes with the safe axonal transport and prevention of intracellular AVP proteolysis.

NPII is a cysteine-rich protein that contains seven disulfide bonds between its 14 cysteine residues. The disulfide bonds occur between codons 41–85, 44–58, 52–75, 59–65, 92–104, 98–116, and 105–110 of the prepro-AVP–NPII sequence. Mutations involving cysteine residues, which form disulfide bonds in the NPII protein, have been described: one in the fourth, three in the fifth, and four in the sixth, and three in the seventh [17, 19]. In the three-dimensional structure of neurophysin, the mutation (c.193T>A) disrupted the normal disulfide bond linking C59 with C65. Until now, Cys65Phe and other mutations involving cysteine residues in four of the seven disulfide bridges have reportedly disrupted the disulfide bonds during protein folding in the endoplasmic reticulum, leading to destabilization of protein structure and function in NPII [19, 20]. The novel mutation involving the disruption of the fourth disulfide bond in the AVP–NPII precursor protein will enable us to further investigate the pathogenesis of adFNDI, at the molecular level.

Mutations in the NPII-coding region are apparently linked with an early disease onset. Interestingly, fewer cases live to a normal age as reported here, beyond the first few decades, possibly due to adequate AVP to maintain the urine volume. A few researchers report that the pathogenesis may be associated with abnormal neuronal secretion due to improper AVP protein folding or synthesis compared

with individuals possessing the normal alleles. Furthermore, the cadaver studies of ADNDI patients show that the neurocytes in the patients' hypothalamus for AVP have selective deletion. Other autopsy studies demonstrated that although the structure of hypothalamus and the staining of supraoptic nucleus are normal, the staining of the paraventricular nuclei is abnormal [6, 10, 21–24].

In conclusion, a novel missense mutation identified in the *AVP–NPII* gene resulted in neurohypophyseal DI due to disruption of the fourth disulfide bond in the AVP–NPII precursor protein required for correct folding of the neurophysin moiety; a synonymous mutation (c.192C>A) was identified in the *AVP–NPII* gene in this Chinese family. Although the findings do not directly explain the underlying pathogenesis of the adFNDI, this mutation increases the number of genetic abnormalities in the *AVP–NPII* gene and provides a molecular basis for understanding the characteristics of NPII that are associated with abnormal protein translation.

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References

1. J.H. Christensen, C. Siggaard, S. Rittig, Autosomal dominant familial neurohypophyseal diabetes insipidus. APMIS **109**(Suppl), 92–95 (2003)
2. C.M. Hedrich, A. Zachurzok-Buczynska, A. Gawlik, S. Russ, G. Hahn, K. Koehler, E. Malecka-Tendera, A. Huebner, A autosomal dominant neurohypophyseal diabetes insipidus in two families. Horm. Res. **71**, 111–119 (2009)
3. T. Mary Fujiwara, G. Daniel, Molecular biology of hereditary diabetes insipidus. J. Am. Soc. Nephrol. **16**, 2836–2846 (2005)
4. Y.W. Lee, K.W. Lee, J.W. Ryu, J.O. Mok, C.S. Ki, H.K. Park, Y.J. Kim, S.J. Kim, D.W. Byun, K.I. Suh, M.H. Yoo, H.B. Shin, Y.K. Lee, C.H. Kim, 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. **34**, 12–14 (2008)
5. S. Rittig, G.L. Robertson, C. Siggaard, L. Kovács, N. Gregersen, J. Nyborg, E.B. Pedersen, 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 (1996)
6. J.H. Christensen, C. Siggaard, T.J. Corydon, G.L. Robertson, N. Gregersen, L. Bolund, S. Rittig, Impaired trafficking of mutated AVP prohormone in cells expressing rare disease genes causing autosomal dominant familial neurohypophyseal diabetes insipidus. Clin. Endocrinol. **60**, 125–136 (2004)
7. H. Mizuno, Y. Sugiyama, Y. Ohro et al., Clinical characteristics of eight patients with congenital nephrogenic diabetes insipidus. Endocrine **24**(1), 55–59 (2004)
8. N. Skordis, P.C. Patsalis, J.A. Hettinger, M. Kontou, E. Herakleous, M.R. Krishnamani, J.A. Phillips 3rd, A novel arginine vasopressin–neurophysin II mutation causes autosomal dominant neurohypophyseal diabetes insipidus and morphologic pituitary changes. Horm. Res. **53**, 239–245 (2000)
9. J. Rutishauser, P. Kopp, M.B. Gaskill, T.J. Kotlar, G.L. Robertson, Brief communication: a novel mutation (R97C) in the neurophysin moiety of prepro- vasopressin–neurophysin II associated with autosomal-dominant neurohypophyseal diabetes insipidus. Mol. Genet. Metab. **67**, 89–92 (1999)
10. S. Rittig, C. Siggaard, M. Ozata, I. Yetkin, N. Gregersen, E.B. Pedersen, G.L. Robertson, Autosomal dominant neurohypophyseal diabetes insipidus due to substitution of histidine for tyrosine (2) in the vasopressin moiety of the hormone precursor. J. Clin. Endocrinol. Metab. **87**, 3351–3355 (2002)
11. G. Olias, D. Richter, H. Schmale, Heterologous expression of human vasopressin–neurophysin precursors in a pituitary cell line: defective transport of a mutant protein from patients with familial diabetes insipidus. DNA Cell. Biol. **15**, 929–935 (1996)
12. C. Siggaard, S. Rittig, T.J. Corydon, P.H. Andreasen, T.G. Jensen, B.S. Andresen, G.L. Robertson, N. Gregersen, L. Bolund, E.B. Pedersen, Clinical and molecular evidence of abnormal processing and trafficking of the vasopressin preprohormone in a large kindred with familial neurohypophyseal diabetes insipidus due to a signal peptide mutation. J. Clin. Endocrinol. Metab. **84**, 2933–2944 (1999)
13. P.C. Gagliardi, S. Bernasconi, D.R. Repaske, Autosomal dominant neurohypophyseal diabetes insipidus associated with a missense mutation encoding Gly233Valin neurophysin II. J. Clin. Endocrinol. Metab. **82**, 3643–3646 (1997)
14. J. Rutishauser, M. Boni-Schnetzler, J. Böni, W. Wichmann, T. Huisman, M.B. Valloton, E.R. Froesch, A novel point mutation in the translation initiation codon of the pre-pro-vasopressin–neurophysin II gene: cosegregation with morphological abnormalities and clinical symptoms in autosomal dominant neurohypophyseal diabetes insipidus. J. Clin. Endocrinol. Metab. **81**, 192–198 (1996)
15. J. Birk, M.A. Friberg, C. Prescianotto-Baschong, M. Spiess, J. Rutishauser, Dominant pro-vasopressin mutants that cause diabetes insipidus form disulfide-linked fibrillar aggregates in the endoplasmic reticulum. J. Cell. Sci. **122**, 3994–4002 (2009)
16. J.R. Green, G.C. Buchan, E.C. Alvord Jr, A.G. Swanson, Hereditary and idiopathic types of diabetes insipidus. Brain **90**, 707–714 (1967)
17. C.E. Flück, J. Deladoëy, S. Nayak, O. Zeller, P. Kopp, P.E. Mullis, Autosomal dominant neurohypophyseal diabetes insipidus in a Swiss family, caused by a novel mutation(C59D/A60W) in the neurophysin moiety of prepro-vasopressin–neurophysin II, (AVP–NPII). Eur. J. Endocrinol. **145**, 439–444 (2001)
18. H. Kobayashi, I. Fujisawa, K. Ikeda, C. Son, T. Iwakura, A. Yoshimoto, M. Kasahara, T. Ishihara, Y. Ogawa, A novel heterozygous missense mutation in the vasopressin moiety is identified in a Japanese person with neurohypophyseal diabetes insipidus. J. Endocrinol. Invest. **29**, 252–256 (2006)
19. S. Baglioni, G. Corona, M. Maggi, M. Serio, A. Peri, Identification of a novel mutation in the arginine vasopressin–neurophysin II gene affecting the sixth intrachain disulfide bridge of the neurophysin II moiety. Eur. J. Endocrinol. **151**, 605–611 (2004)
20. A.P. Abbes, H. Engel, E.J. Bruggeman, A.A. Franken, Human gene mutations. Hum. Genet. **118**, 774–785 (2006)
21. M. Ozata, C. Tayfun, K. Kurtaran, I. Yetkin, Z. Beyhan, A. Corakci, S. Cağlayan, A. Alemdaroglu, M.A. Gündogan, Magnetic resonance imaging of posterior pituitary for evaluation of the neurohypophyseal function in idiopathic and autosomal dominant neurohypophyseal diabetes insipidus. Eur. Radiol. **7**, 1098–1102 (1997)

22. G. Boutzios, S. Livadas, E. Marinakis et al., Endocrine and metabolic aspects of the Wolfram syndrome. Endocrine **40**(1), 10–13 (2011)
23. C. Bergeron, K. Kovacs, C. Ezrin, C. Mizzen, Hereditary diabetes insipidus: an immunohistochemical study of the hypothalamus and pituitary gland. Acta Neuropathol. **81**, 345–348 (1991)
24. C.P. Mahoney, E. Weinberger, C. Bryant, M. Ito, J.L. Jameson, M. Ito, Effects of aging on vasopressin production in a kindred with autosomal dominant neurohypophyseal diabetes insipidus due to the DeltaE47 neurophysin mutation. J. Clin. Endocrinol. Metab. **87**, 870–876 (2002)