

NOTE

Novel Mutation of *Aquaporin-2* Gene in a Patient with Congenital Nephrogenic Diabetes Insipidus

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Abstract. Congenital nephrogenic diabetes insipidus (CNDI) is a rare inherited disease, characterized by an inability of the kidney to concentrate urine in response to vasopressin. Three different inheritance patterns have been described, i.e., the X-linked recessive form associated with arginine vasopressin V2 receptor (*AVPR2*) gene mutations, the autosomal recessive and dominant forms of CNDI associated with mutations in the aquaporin-2 (*AQP2*) gene encoding the vasopressin-regulated water channel of the renal collecting duct. Our case is an 18-year-old male patient who complained of severe polyuria since his infancy. But his developmental and growth status were normal. He was diagnosed as CNDI by water deprivation test and genomic DNA sequencing, which revealed high plasma AVP levels but persistently low urine osmolalities to 6 h-water deprivation and the novel missense mutation S216F in exon4 of the *AQP2* gene. Immunohistochemistry of renal biopsied tissue revealed that most of the *AQP2* labeling was seen intracellularly in a dotted pattern in the collecting duct principal cells. Immunoblotting of urine samples revealed significantly decreased urinary excretion of *AQP2* (~7% of normal control). Here, we report a new case of CNDI associated with the novel missense mutation of the *AQP2* gene.

Key words: Congenital nephrogenic diabetes insipidus, Aquaporin-2, Polyuria, Missense mutation, Renal biopsy

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CONGENITAL nephrogenic diabetes insipidus is a rare inherited disorder characterized by an inability to concentrate urine in response to the antidiuretic effect of vasopressin [1]. The disorder causes polyuria of more than 3 liters per day, nocturia, functional obstruction, hypernatremia and compensatory polydipsia. Patients with this disorder exhibit hypernatremic dehydration, failure to thrive, irritability, and fever during infancy. The disorder is known to be caused by the mutations of *AVPR2* gene or *AQP2* gene. The former is associated with X-linked recessive form and the

latter is associated with autosomal recessive or dominant inheritance [2-4]. About 10 % of CNDI might be caused by *AQP2* gene mutations, and most of them are autosomal recessive forms [1]. The gene for human *AQP2* has been localized to chromosomal region 12q13 [3]. So far, various mutations in the *AQP2* gene have been demonstrated in both the autosomal recessive and dominant forms of NDI, in which mutations are distributed throughout the gene without any mutation hot spot [5-10]. Here, we report a Korean male patient with CNDI caused by the novel missense mutation S216F in exon4 of the *AQP2* gene.

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Case Report

An 18-year-old man presented with severe polyuria which had occurred since his infancy. His prenatal

Table 1. Dehydration Test Results of the Patient

	0hr	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr
UVol (mL)	700	550	500	500	600	550	500	350	300
BWt (Kg)	67.7	67.2	66.4	65.8	65.2	64.4	63.8	63.1	62.5
Uosm (mOsm/KgH ₂ O)	60	58	58	58	60	62	66	74	76
Posm (mOsm/KgH ₂ O)	285	291	291	300	300	305	303	314	313
Na/K (mmol/L)	145/4.7	144/4.7	146/4.6	151/5.3	151/4.7	150/4.8	153/4.8	154/5.5	154/4.8
BP (SBP/DBP) (mmHg)	131/76	121/80	130/77	135/67	132/55	123/67	107/61	106/54	103/54

UVol, urine volume; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; BWt, body weight; Uosm, urine osmolality; Posm, plasma osmolality; Na/K, serum sodium concentration/serum potassium concentration.

and natal history was unremarkable. He had no family history of NDI, and his parents were not consanguineous. He had polyuria, nocturia, an eager for water intake, and recurrent episodes of fever since his infancy. Unless he drank enough water, he got nervous and irritable. He took herbal medication several times but it was not effective. His height was 172 cm and body weight was 67 kg. His developmental status was normal mentally and physically. On admission, blood pressure was 123/69 mmHg and pulse rate was 76 beats/min. Daily urine output was more than 10 liters and urine specific gravity was 1.005. Urine osmolality and plasma osmolality were 58 mOsm/KgH₂O and 299 mOsm/KgH₂O, respectively. Serum sodium concentration was 146 mM/L, potassium concentration was 3.8 mM/L. Blood urea nitrogen and creatinine levels were 6.4 mg/dL and 0.89 mg/dL, respectively. Plasma antidiuretic hormone level was 8.39 pg/ml (normal range: 0 - 6.7 pg/dL). During 6-hour of water deprivation test, he lost 3.9 kg of body weight with a drop of 18 mmHg in mean arterial blood pressure, but urine osmolality was unchanged (between 58 and 66 mOsm/kgH₂O), indicating a failure to concentrate urine (Table 1). Importantly, despite parenteral administration of dDAVP (5 U), urine osmolality was unchanged, compatible with nephrogenic diabetes insipidus (Table 1). Brain magnetic resonance imaging showed no abnormality, and ultrasonographic examination of both kidneys revealed no evidence of obstructive nephropathy including hydronephrosis. With his long sustained history since infancy, we considered congenital nephrogenic diabetes insipidus as a tentative diagnosis. Immunoblotting of his urine sample

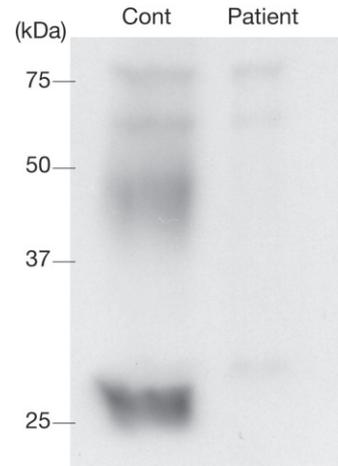


Fig.1. Immunoblotting of AQP2 in urine sample. Urinary excretion of AQP2 in the patient was decreased compared with the control (Cont).

collected for 24 hours revealed markedly decreased AQP2 expression (approximately 7%), compared to the urine sample of normal control volunteer (Fig. 1). Immunofluorescence and electron microscopy of renal biopsied tissue revealed a trace of the IgA deposition in the glomerulus without mesangial hypercellularity (not shown). Immunoperoxidase labeling of AQP2 showed that apical AQP2 labeling in the cortical and outer medullary collecting duct was very sparse and most of the AQP2 labeling was in the cytoplasm in a dotted or speckled pattern (Fig. 2). In contrast, AQP2 labeling in the control kidney from a patient with IgA nephropathy showed apical labeling in the collecting duct. With such findings, he was clinically diagnosed as CNDI, and the *AQP2* gene analysis was

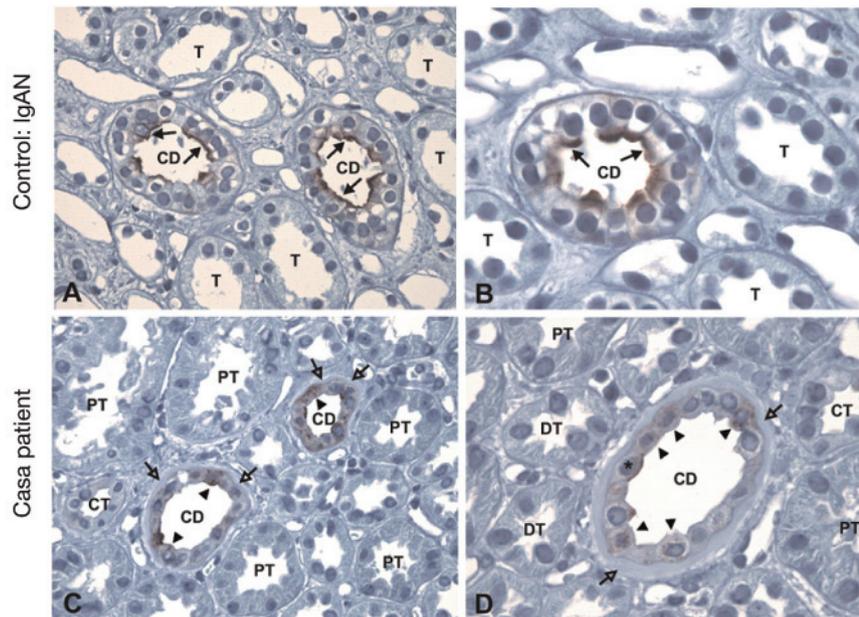


Fig.2. Immunohistochemistry of AQP2 in control kidney from IgA nephropathy (IgAN) and kidney from patient. In panel A and B, abundant AQP2 labeling (arrows) was seen at the apical plasma membrane domain of the outer medullary collecting duct principal cells in the kidney from a control patient with IgAN. Panel C and D showed the kidney sections from the patient of CNDI in this case. Apical AQP2 labeling in the cortical (C and D) and outer medullary (not shown) collecting duct principal cells was sparse (indicated by asterisk) and most of the AQP2 labeling was seen in a dotted or speckled pattern intracellularly (indicated by arrowheads). In addition, thick fibrotic band-like structure (open arrows) was seen around the cortical collecting ducts, whereas it was not associated with other segments of kidney tubule. CD, collecting duct; CT, connecting tubule; DT, distal convoluted tubule; T, thick ascending limb.

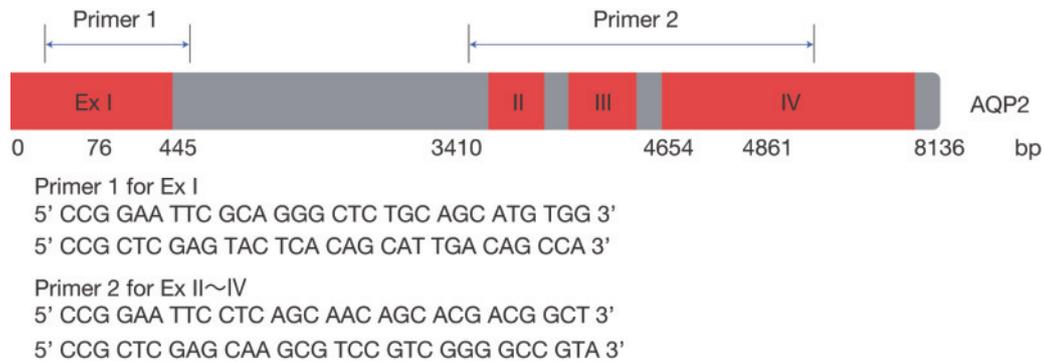


Fig.3. Exon position of individual primer set.

performed. Genomic DNA was extracted from the nucleated cells in the peripheral blood using a commercial kit (Puregene DNA isolation kit, Gentra system, Minneapolis). ORF (open reading frame) in exon 1 and ORF in exon 2 - 4 with unilateral flanking intron of the *AQP2* gene was amplified separately from the genomic DNA by polymerase chain reaction (PCR, Fig. 3), and the amplified exon1 and exon 2 - 4 were inserted to the vector (pBluescript II SK) to be cloned

and were sequenced (MACROGEN INC, Seoul, Korea). The sequences of the PCR primers were as follows; exon 1, sense 5'-CCG GAA TTC GCA GGG CTC TGC AGC ATG TGG-3', antisense 5'-CGC CTC GAG TAC TCA CAG CAT TGA CAG CCA-3'; exon 2 - 4, sense 5'-CCG GAA TTC CTC AGC AAC AGC ACG ACG GCT-3', antisense 5'-CCG CTC GAG CAA GCG TCC GTC GGG GCC GTA-3' (Fig. 3). One heterogeneous missense point, ²¹⁶Ser(TCC) to Phe(TTC) in exon

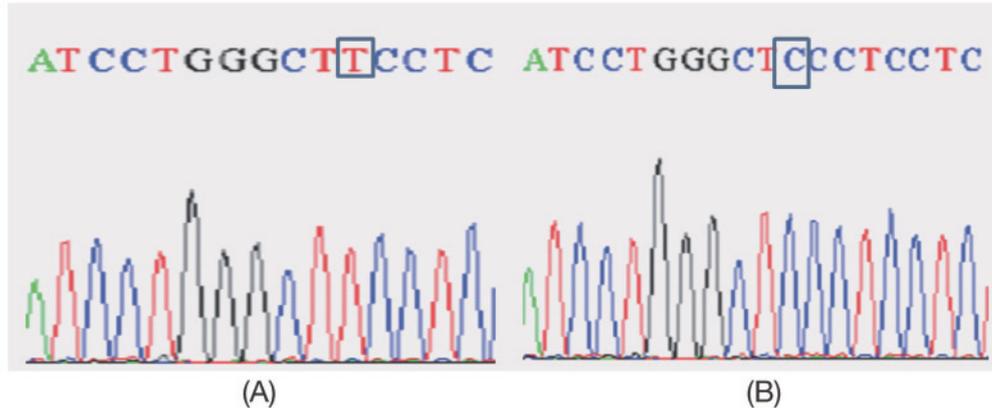


Fig.4. (A) DNA sequence with a mutated allele of the patient. The small square indicates the site of point mutation (B) DNA sequence with normal allele of the patient. The small square indicates the corresponding normal site.

4 of the *AQP2* gene was detected in the patient (Fig. 4). That is located on the transmembrane domain 6 of the *AQP2*. He was treated with hydrochlorothiazide and amiloride, but his polyuria was only partially relieved.

Discussion

AQP2 is the AVP-dependent water channel of the collecting duct [11-14]. In the presence of AVP, solute-free water is reabsorbed osmotically through the principal cells of the collecting ducts, resulting in the excretion of concentrated urine. This antiuretic effect is mediated via a G protein-coupled V2 receptor that accumulates intracellular cyclic AMP and, increases protein kinase A activity. This lead to phosphorylation of *AQP2*, particularly on its Ser256 residue [15-17], resulting in translocation of *AQP2* water channels into the apical membrane [18].

About 10% of patients diagnosed as CNDI have mutations in the *AQP2* gene. Deen *et al.* [3] found mutated and non-functional *AQP2* in patients with severe nephrogenic diabetes insipidus (non-X-linked NDI). The patient appeared to carry two point mutations in the *AQP2* gene, one resulting in substitution of a cysteine for arginine 187 (R187C) in the third extracellular loop of the *AQP2* and the other resulting in substitution of a proline for serine 216 (S216P) in the sixth transmembrane domain [3]. The patient was diagnosed at 3 months after birth. In contrast, our patient had one heterogenous missense point, ²¹⁶Ser(TCC) to Phe(TTC) in exon 4 of the *AQP2* gene, which has not been reported previously, to our knowl-

edge. Our patient had only a missense mutation in exon 4 of the *AQP2* gene and was diagnosed at age 18, although his symptoms had begun to develop since infancy. Ultrasonographic findings of his kidneys did not reveal evidence of obstructive nephropathy including hydronephrosis, suggesting that his clinical manifestation was not as severe as the usual features seen in autosomal recessive form of CNDI [19, 20]. *AQP2* proteins are usually not detected in the urine of patients suffering from autosomal recessive CNDI as a result of *AQP2* gene mutation [21]. In the urine of our patient, however, *AQP2* proteins were weakly (~ 7% of normal control) detected by immunoblotting. Moreover, immunohistochemistry of *AQP2* in the patient's kidney showed sparse but distinct apical *AQP2* labeling in the cortical collecting duct, although most of the *AQP2* labeling was observed intracellularly in a dotted or speckled pattern indicating a defect in the vasopressin action on *AQP2* trafficking and retention of *AQP2* in certain subcellular compartment. Expression of the mutant S216P *AQP2* protein in *Xenopus* oocytes revealed a nonfunctional water channel caused by an impaired routing to the plasma membrane [22]. Therefore, the mutant S216F *AQP2* is inferred to have a severe impairment in trafficking to the apical membrane. Since patients with autosomal dominant mutations of *AQP2* gene are likely to demonstrate more mild clinical manifestations than recessive form [5, 19, 20, 23], the CNDI seen in this case is likely to be autosomal dominant form rather than recessive form. All mutations in dominant CNDI are found in the coding region of the C-terminal tail of *AQP2*, which plays an important role in *AQP2* traf-

ficking to the apical plasma membrane. Thus, AQP2 mutants in dominant NDI are sorted to other subcellular locations in the cells than wt-AQP2 [19, 20, 24, 25]. In contrast, this patient's missense mutation was in the transmembrane domain 6 of the AQP2, which AQP2 mutant needs to be examined in further study.

So far, renal histology in patients with CNDI has rarely been performed, since hydronephrosis is commonly accompanied, which limits the procedure of renal biopsy. Hironak, *et al.* [26] examined the kidney tissue of a 16-year-old male NDI patient which was normal except for a minor abnormality in the section of the tubule closest to the glomerulus. In this patient, except the changes of AQP2 labeling, thick fibrotic band-like structure around the cortical collecting ducts and scanty deposition of IgA in the glomerulus were seen, but the specificity of both findings in CNDI could not be explored further. In addition, we could not perform his parents' AQP2 gene analysis due to their privacy issues, and thus the inheritance pattern

for this case could not be presented.

In summary, we here report a case of male patient with CNDI with severe polyuria and a novel missense mutation ²¹⁶Ser(TCC) to Phe(TTC) in exon 4 of the AQP2 gene, which is associated with markedly decreased apical AQP2 expression in the collecting duct principal cells proven by immunohistochemistry and decreased urinary excretion of AQP2 demonstrated by urine immunoblotting.

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References

- 1 Knoers, N.V. and Deen, P.M. (2001) Molecular and cellular defects in nephrogenic diabetes insipidus. *Pediatr Nephrol* 16, 1146-52.
- 2 Rosenthal, W., Seibold, A., Antaramian, A., Lonergan, M., Arthus, M.F., Hendy, G.N., Birnbaumer, M. and Bichet, D.G. (1992) Molecular identification of the gene responsible for congenital nephrogenic diabetes insipidus. *Nature* 359, 233-5.
- 3 Deen, P.M., Verdijk, M.A., Knoers, N.V., Wieringa, B., Monnens, L.A., van Os, C.H. and van Oost, B.A. (1994) Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science* 264, 92-5.
- 4 van Lieburg, A.F., Verdijk, M.A., Knoers, V.V., van Essen, A.J., Proesmans, W., Mallmann, R., Monnens, L.A., van Oost, B.A., van Os, C.H. and Deen, P.M. (1994) Patients with autosomal nephrogenic diabetes insipidus homozygous for mutations in the aquaporin 2 water-channel gene. *Am J Hum Genet* 55, 648-52.
- 5 Loonen, A.J., Knoers, N.V., van Os, C.H. and Deen, P.M. (2008) Aquaporin 2 mutations in nephrogenic diabetes insipidus. *Semin Nephrol* 28, 252-65.
- 6 Robben, J.H., Knoers, N.V. and Deen, P.M. (2006) Cell biological aspects of the vasopressin type-2 receptor and aquaporin 2 water channel in nephrogenic diabetes insipidus. *Am J Physiol Renal Physiol* 291, F257-70.
- 7 Tajima, T., Okuhara, K., Satoh, K., Nakae, J. and Fujieda, K. (2003) Two novel aquaporin-2 mutations in a sporadic Japanese patient with autosomal recessive nephrogenic diabetes insipidus. *Endocr J* 50, 473-6.
- 8 Bircan, Z., Karacayir, N. and Cheong, H.I. (2008) A case of aquaporin 2 R85X mutation in a boy with congenital nephrogenic diabetes insipidus. *Pediatr Nephrol* 23, 663-5.
- 9 Mulders, S.M., Knoers, N.V., Van Lieburg, A.F., Monnens, L.A., Leumann, E., Wuhl, E., Schober, E., Rijss, J.P., Van Os, C.H. and Deen, P.M. (1997) New mutations in the AQP2 gene in nephrogenic diabetes insipidus resulting in functional but misrouted water channels. *J Am Soc Nephrol* 8, 242-8.
- 10 Lin, S.H., Bichet, D.G., Sasaki, S., Kuwahara, M., Arthus, M.F., Lonergan, M. and Lin, Y.F. (2002) Two novel aquaporin-2 mutations responsible for congenital nephrogenic diabetes insipidus in Chinese families. *J Clin Endocrinol Metab* 87, 2694-700.
- 11 Fushimi, K., Uchida, S., Hara, Y., Hirata, Y., Marumo, F. and Sasaki, S. (1993) Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature* 361, 549-52.
- 12 Nielsen, S., Chou, C.L., Marples, D., Christensen, E.I., Kishore, B.K. and Knepper, M.A. (1995) Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. *Proc Natl Acad Sci U S A* 92, 1013-7.
- 13 Kwon, T.H., Nielsen, J., Moller, H.B., Fenton, R.A.,

- Nielsen, S. and Frokiaer, J. (2009) Aquaporins in the kidney. *Handb Exp Pharmacol* 95-132.
- 14 Nielsen, S., Frokiaer, J., Marples, D., Kwon, T.H., Agre, P. and Knepper, M.A. (2002) Aquaporins in the kidney: from molecules to medicine. *Physiol Rev* 82, 205-44.
- 15 Fushimi, K., Sasaki, S. and Marumo, F. (1997) Phosphorylation of serine 256 is required for cAMP-dependent regulatory exocytosis of the aquaporin-2 water channel. *J Biol Chem* 272, 14800-4.
- 16 Katsura, T., Gustafson, C.E., Ausiello, D.A. and Brown, D. (1997) Protein kinase A phosphorylation is involved in regulated exocytosis of aquaporin-2 in transfected LLC-PK1 cells. *Am J Physiol* 272, F817-22.
- 17 Linshaw, M.A. (2007) Back to basics: congenital nephrogenic diabetes insipidus. *Pediatr Rev* 28, 372-80.
- 18 Christensen, B.M., Zelenina, M., Aperia, A. and Nielsen, S. (2000) Localization and regulation of PKA-phosphorylated AQP2 in response to V(2)-receptor agonist/antagonist treatment. *Am J Physiol Renal Physiol* 278, F29-42.
- 19 Kamsteeg, E.J., Bichet, D.G., Konings, I.B., Nivet, H., Lonergan, M., Arthus, M.F., van Os, C.H. and Deen, P.M. (2003) Reversed polarized delivery of an aquaporin-2 mutant causes dominant nephrogenic diabetes insipidus. *J Cell Biol* 163, 1099-109.
- 20 Kuwahara, M., Iwai, K., Ooeda, T., Igarashi, T., Ogawa, E., Katsushima, Y., Shinbo, I., Uchida, S., Terada, Y., Arthus, M.F., Lonergan, M., Fujiwara, T.M., Bichet, D.G., Marumo, F. and Sasaki, S. (2001) Three families with autosomal dominant nephrogenic diabetes insipidus caused by aquaporin-2 mutations in the C-terminus. *Am J Hum Genet* 69, 738-48.
- 21 Kanno, K., Sasaki, S., Hirata, Y., Ishikawa, S., Fushimi, K., Nakanishi, S., Bichet, D.G. and Marumo, F. (1995) Urinary excretion of aquaporin-2 in patients with diabetes insipidus. *N Engl J Med* 332, 1540-5.
- 22 Deen, P.M., Croes, H., van Aubel, R.A., Ginsel, L.A. and van Os, C.H. (1995) Water channels encoded by mutant aquaporin-2 genes in nephrogenic diabetes insipidus are impaired in their cellular routing. *J Clin Invest* 95, 2291-6.
- 23 Marr, N., Bichet, D.G., Lonergan, M., Arthus, M.F., Jeck, N., Seyberth, H.W., Rosenthal, W., van Os, C.H., Oksche, A. and Deen, P.M. (2002) Heterologomerization of an Aquaporin-2 mutant with wild-type Aquaporin-2 and their misrouting to late endosomes/lysosomes explains dominant nephrogenic diabetes insipidus. *Hum Mol Genet* 11, 779-89.
- 24 Procino, G., Carmosino, M., Marin, O., Brunati, A.M., Contri, A., Pinna, L.A., Mannucci, R., Nielsen, S., Kwon, T.H., Svelto, M. and Valenti, G. (2003) Ser-256 phosphorylation dynamics of Aquaporin 2 during maturation from the ER to the vesicular compartment in renal cells. *Faseb J* 17, 1886-8.
- 25 de Mattia, F., Savelkoul, P.J., Bichet, D.G., Kamsteeg, E.J., Konings, I.B., Marr, N., Arthus, M.F., Lonergan, M., van Os, C.H., van der Sluijs, P., Robertson, G. and Deen, P.M. (2004) A novel mechanism in recessive nephrogenic diabetes insipidus: wild-type aquaporin-2 rescues the apical membrane expression of intracellularly retained AQP2-P262L. *Hum Mol Genet* 13, 3045-56.
- 26 Hironaka, K., Makino, H., Ogura, T. and Ota, Z. (1995) Renal histology in a patient with nephrogenic diabetes insipidus. *Nephron* 71, 224-6.