Aquaporin 2 Mutations in Nephrogenic Diabetes Insipidus

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Summary: Water reabsorption in the renal collecting duct is regulated by the antidiuretic hormone vasopressin (AVP). When the vasopressin V2 receptor, present on the basolateral site of the renal principal cell, becomes activated by AVP, aquaporin-2 (AQP2) water channels will be inserted in the apical membrane, and in this fashion, water can be reabsorbed from the pro-urine into the interstitium. The essential role of the vasopressin V2 receptor and AQP2 in the maintenance of body water homeostasis became clear when it was shown that mutations in their genes cause nephrogenic diabetes insipidus, a disorder in which the kidney is unable to concentrate urine in response to AVP. This review describes the current knowledge on AQP2 mutations in nephrogenic diabetes insipidus.
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The mammalian kidney has the ability to excrete or reabsorb free water independent of changes in solute excretion. Physiologic studies have provided evidence for the constitutive absorption of approximately 90% of the glomerular filtration rate volume in the proximal tubules and descending limbs of Henle's loop, whereas facultative water reabsorption occurs in the collecting ducts under control of the antidiuretic hormone arginine vasopressin (AVP). Although all cell membranes have a surprisingly high diffusive water permeability, there is now extensive evidence that water transport in the nephron is facilitated by water channels.[1] Because water movement across cell membranes is essential for all living organisms, it is no surprise that the initial cloning of aquaporin (*AQP*) 1 has reanimated research into the biology of water transport and mammalian osmoregulation. Water balance is regulated by the combined action of osmoreceptors, volume receptors, thirst sensation, vasopressin, and water excretion or reabsorption by the kidney. To date, 13 mammalian AQPs have been identified (AQP0-12). Functional studies have identified a subgroup of AQPs (AQPs 3, 7, and 9), termed *aquaglyceroporins*, that transport water as well as glycerol and possibly urea and other small solutes.[2-4] AQPs have 6 transmembrane domains, connected by 5 loops (A-E), and NH2 and COOH-termini that are located in the cytoplasm (Fig. 1). The AQP family shows a highly conserved asparagine-proline-alanine (NPA) motif in both loops B and E, which fold back into the membrane in an hourglass fashion to form the water pore. Although AQPs form tetrameric complexes, each single subunit is able to form an aqueous pore and is able to transport water.

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AQP2

AQP2 is the AVP-dependent water channel of the collecting duct (for review see Knepper and Inoue[5]). It is well established that the collecting duct water permeability can be regulated in 2

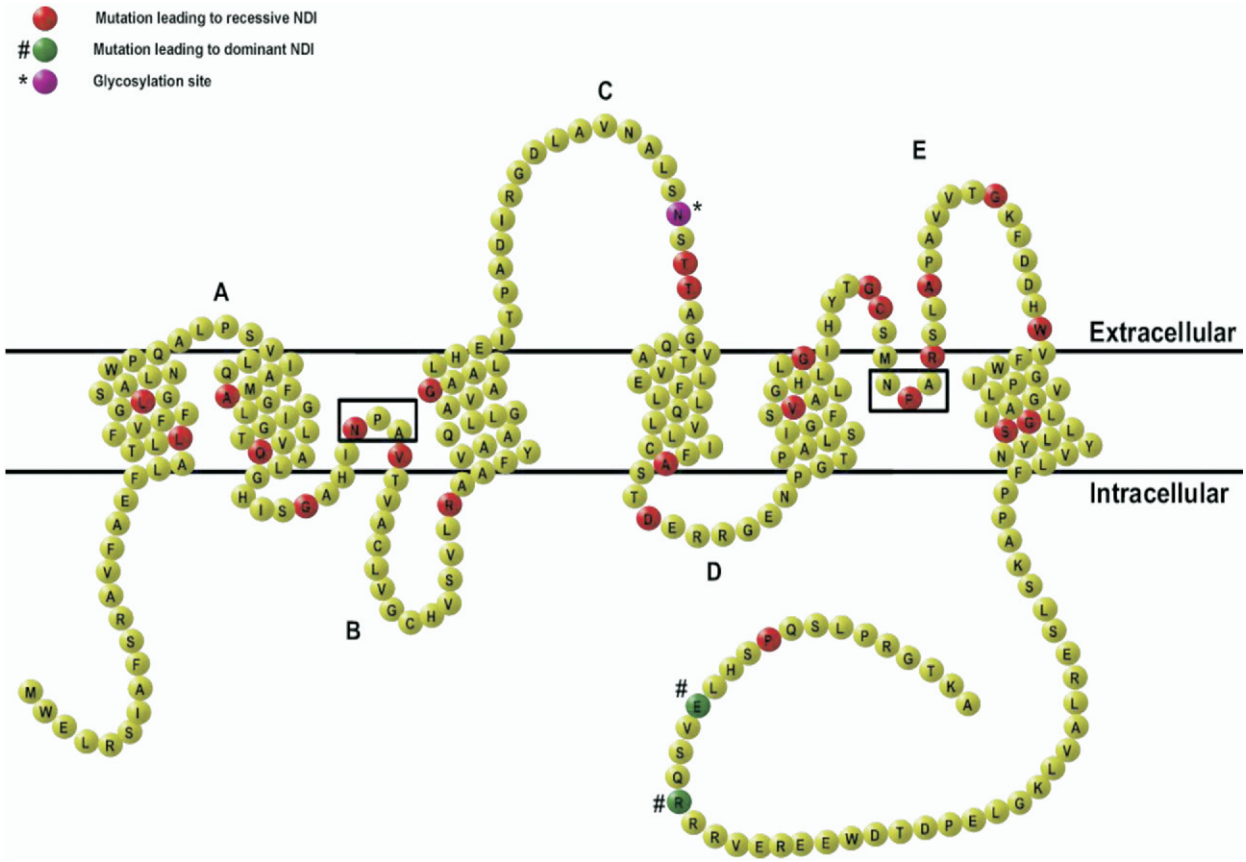

Figure 1. AQP2 structure. An AQP monomer consists of 6 transmembrane domains connected by 5 loops (A through E) and its N- and C-terminus located intracellularly. Loops B and E meet each other with their characteristic NPA (Asparagine-Proline-Alanine) boxes (black rectangle) in the membrane and form the water selective pore. The N-glycosylation site (pink; *), the AQP2 mutations leading to recessive NDI (red) and dominant NDI (green; #) are indicated.

ways: a short-term regulation, which occurs in minutes, and a long-term regulation, which takes hours.

In the short-term regulation, AVP is released upon states of hypovolemia or hypernatremia from the posterior pituitary gland into the bloodstream and is transported to the kidneys, where it can bind to vasopressin V2 receptors located on the basolateral side of collecting duct principal cells.[6] This hormone binding results in the switch of a vasopressin type 2 receptor (V2R)-bound guanosine diphosphate–coupled trimeric Gs protein to its active guanosine triphosphate–bound form, which subsequently dissociates into its α- and $\beta\gamma$-subunits. The activated V2R triggers a signaling cascade in which membrane-bound adenylyl cyclase is stimulated by the α-subunit of the trimeric Gs protein to convert adenosine triphosphate to cyclic adenosine monophosphate (cAMP), cAMP levels in the cell subsequently increase, which leads to an increase in protein kinase A activity and to phosphorylation of AQP2 water channels on its Ser256 residue,[7,8] resulting in its steady-state redistribution from intracellular vesicles to the apical membrane. After an osmotic gradient of sodium and urea, water will enter the cell via AQP2 and leave the cell on the basolateral side via AQP3 and AQP4.[9–11] Fig. 2 shows an overview of this process. Phosphorylation of Ser256 is essential for the translocation of AQP2 to the apical membrane, although it does not alter the water permeability of AQP2.[12] It has been shown that the AQP2-S256A mutant, which mimics a constitutively nonphosphorylated AQP2 protein, is retained in intracellular vesicles, independent of the presence of the adenylate cyclase activator, forskolin.[7,8,13,14] Interesting in this respect is that in an unstimulated steady-state con-

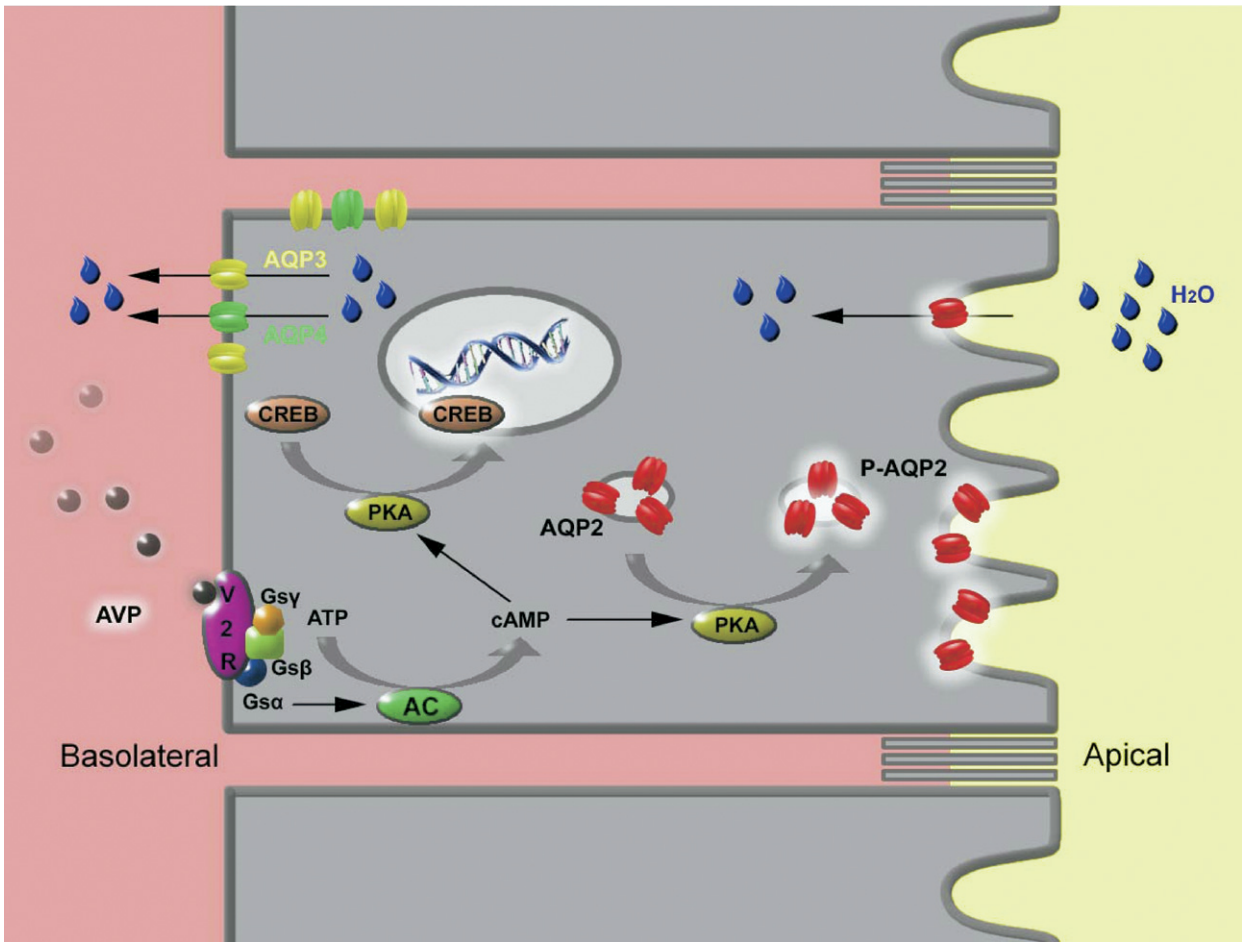

Figure 2. Regulation of AQP2-mediated water transport by vasopressin. AVP, V2R, stimulatory guanosine triphosphate binding protein (Gsα protein), adenylate cyclase (AC), adenosine triphosphate (ATP), cAMP, protein kinase A (PKA), phosphorylated AQP2 (P-AQP2), and phosphorylated cAMP response element–binding protein (glowing CREB) are indicated. For details see text.

dition, phosphorylated AQP2 could be detected in intracellular vesicles in the kidney.[15] AQP2 is expressed as a homotetramer[13,16] and studies using oocytes as a model system indicated that for a plasma membrane localization 3 out of 4 monomers in an AQP2 tetramer need to be phosphorylated.[17]

Upon fluid intake, AVP release into the blood decreases, AQP2 is redistributed into intracellular vesicles, and water reabsorption is reduced.[18–21] Katsura et al[22] have shown that the AVP-regulated recycling of AQP2 can occur at least 6 times with the same molecules.

In the long-term regulation, circulating AVP levels increase collecting duct water permeability by increasing the expression level of AQP2.[23,24] Different cis-acting elements have been reported to be located in the promoter region of the *AQP2* gene and of these it was shown that they were able to confer AVP-induced expression of a reporter gene.[25,26] Transcriptional regulation of the *AQP2* gene seems to be the main action of AVP and is mediated by phosphorylation of a cAMP response element–binding protein and binding of phosphorylated cAMP response element–binding protein to the cAMP response element in the promoter region of the *AQP2* gene.[27]

THE ROLE OF AQP2 IN DISEASES

Several pathophysiologic conditions exist that can lead to a disturbed water homeostasis. Because AVP is the hormone that controls serum osmolality by decreasing free water clearance,

any condition that interferes with AVP production, secretion, and binding to V_2 receptors or with AQP2 synthesis and trafficking will result in loss of the ability to concentrate urine. With central diabetes insipidus, the production of functional AVP is disturbed and therefore patients are not able to concentrate their urine. Numerous mutations in AVP have been reported and can be the cause of central diabetes insipidus.[28–32] However, administration of the synthetic AVP homolog 1-desamino-8-D-arginine vasopressin (DDAVP) usually is able to decrease urine output in these patients.

Besides central diabetes insipidus, a disturbed water balance also can be of renal origin, which consequently is called *nephrogenic diabetes insipidus* (NDI). Acquired NDI is the most common form. Lithium, which is the drug of choice for treating bipolar disorders, is able to reduce depressive symptoms in 70% to 80% of patients and reduces the mortality rate observed in this illness.[33] Development of NDI is an unpleasant side effect of this therapy and because 0.1% of the world population is on lithium therapy and severe forms of this side effect are present in about 10% to 20% of patients, lithium-induced NDI is the most common acquired form of NDI.[34] Conditions of hypokalemia (as is common in hypertensive patients treated with thiazide diuretics), hypercalcemia, and bilateral ureteral obstruction often also lead to acquired forms of NDI, a disease in which the kidney does not respond to AVP. Paradoxically, diseased states such as congestive heart failure, cirrhosis, pre-eclampsia, and the syndrome of inappropriate release of the antidiuretic hormone (SIADH) induce excessive renal water reabsorption, which can lead to life-threatening hyponatremia and edema formation. From numerous rat model studies it has become clear that all these forms of acquired NDI coincide with a strong reduction in expression of *AQP2*, whereas the states of excessive renal water uptake coincide with increased *AQP2* expression (for reviews see Nielsen et al,[21] Schrier et al,[35] and Marples et al[36]). So far, all data collected on AQP2 confirm its unique role, which is AVP-regulated control of the osmotic water permeability of the principal cell by insertion of AQP2 water channels in the apical plasma membrane, which is otherwise extremely water impermeable.

Besides acquired NDI, a congenital form of NDI also exists, which is quite rare and has an occurrence of 1 per 100,000 human beings. Although congenital NDI is rare, it is important to identify it very early in life to prevent dehydration and its consequences. Congenital NDI can be divided into X-linked (V2R) and autosomal-recessive and -dominant NDI (AQP2). The role of the *V2R* gene in NDI is covered by Dr. Daniel Bichet on page 245 in this issue. In this review, we discuss congenital NDI, which results from mutations found in *AQP2*. The discovery of different genetic causes of NDI has important implications for genetic counseling, especially in those families in which only one member is affected.

THE AQP2 GENE

Chromosome 12q13 harbors the human *AQP2* gene (GenBank accession number z29491)[37,38] as part of an aquaporin gene cluster to which *AQP0*, *AQP5*, and *AQP6* also belong. The *AQP2* gene comprises 4 exons distributed over approximately 5 kb of genomic DNA. The 1.5-kb mRNA encodes a protein of 271 amino acids, which has a predicted molecular weight of 29 kd.

IDENTIFICATION OF THE AQP2 GENE AS THE GENE CAUSING AUTOSOMAL-RECESSIVE NDI

An apparently sporadic Dutch male NDI patient provided evidence for the existence of a non–X-linked type of NDI. Careful clinical studies unraveled a clue leading to the elucidation of a new gene defect for NDI. The patient involved could be differentiated from patients with X-linked NDI on the basis of a DDAVP infusion test. DDAVP is a selective agonist for the V2R. In addition to the renal V2R, which mediates the antidiuretic effect of AVP and DDAVP, there is evidence for an extrarenal V2R, which mediates extrarenal effects of these ligands. An increase in factor VIII, von Willebrand factor, and tissue-type plasminogen activator, and a decrease in diastolic blood pressure[39,40] are the result of stimulation of this extrarenal V2R. In

contrast to the blunted coagulation, fibrinolytic, and vasodilatory responses seen in most NDI patients after DDAVP infusion and reflecting a general V2R defect,[41–43] the Dutch male NDI patient showed a normal response to DDAVP.[44] This finding indicated that the unresponsiveness to vasopressin in this boy was restricted to the kidney. The results of the DDAVP test, together with the finding that no deleterious mutations were found in the coding region of the *V2R* gene, suggested a defect in a kidney-specific protein in the cellular vasopressin-signaling cascade beyond the V2R. When Fushimi et al[45] cloned a water channel of the rat renal collecting duct (CD), which later was renamed AQP2, a candidate gene became available. Expression of its mRNA in *Xenopus* oocytes increased osmotic swelling in response to transfer to a hypotonic buffer, suggesting that it encoded the vasopressin-regulated water channel. Cloning and localization studies of the human equivalent *AQP2* gene favored its potential involvement in autosomal NDI because it localized on chromosome 12q13.[37,46] Subsequently, the *AQP2* gene of the Dutch variant NDI patient was sequenced and he appeared to carry 2 point mutations in the *AQP2* gene, one resulting in substitution of a cysteine for arginine 187 (R187C) in the third extracellular loop of the water channel and the other resulting in substitution of a proline for serine 216 (S216P) in the sixth transmembrane domain.[47] The parents of the boy each carried 1 of these 2 missense mutations but showed no signs of concentrating disabilities. Further studies revealed that each mutation resulted in nonfunctional water channels.[47] All together, the findings indicated that aberrant AQP2 water channels underlie autosomal-recessive NDI, an assumption that was strengthened further by the subsequent detection of mutations in other families, in which patients were from consanguineous couples.[48]

DIFFERENCES BETWEEN NDI CAUSED BY AQP2 OR V2R MUTATIONS

No differences in clinical symptoms between X-linked and autosomal-recessive forms of NDI can be observed, nor in the time of onset of the disease, with the exception of a few cases. In a minority of patients with X-linked NDI, namely in those patients who are carrying *V2R* mutations resulting in partial insensitivity to AVP, the disease onset was reported to be not directly after birth, but later in childhood. In general, the initial symptoms in most autosomal-dominant cases also appear later in childhood. After the observation of normal extrarenal responses to DDAVP in the first patient shown to have *AQP2* mutations, it was suggested that intravenous DDAVP administration with measurement of von Willebrand factor, factor VIII, and tissue-type plasminogen activator levels, could be of use in the differentiation between the X-linked and autosomal-recessive forms of NDI. Indeed, it was shown that male patients with autosomal-recessive NDI show normal extrarenal responses to DDAVP, whereas in all studied patients with X-linked NDI these extrarenal responses are absent as a result of an extrarenal mutant vasopressin type 2 receptor.[49] In female patients, the interpretation of this intravenous DDAVP test is more complicated. Although absence of the extrarenal responses to intravenous administration of DDAVP in females clearly points to the presence of a V2 receptor defect, a normal response cannot be interpreted as indicative of a defect beyond the V2 receptor and, thus, of an AQP2 defect.[50] For instance, a symptomatic female patient described by Moses et al,[51] who was shown to be heterozygous for a V2R mutation, showed a 2-fold increase in factor VIII activity after administration of DDAVP. This discrepancy between the extrarenal and renal response to DDAVP in these female V2 receptor mutation carriers may be owing to variability of the pattern of X-chromosome inactivation between different tissues.[52]

AUTOSOMAL-RECESSIVE NDI

About 10% of patients diagnosed with NDI have mutations in the *AQP2* gene. Of these, greater than 90% are diagnosed with recessive NDI. To date, 39 mutations in *AQP2* have been reported (Table 1), of which 32 are involved in recessive NDI. Interestingly, nearly all the mutations in autosomal-recessive NDI are found in the region encoding the AQP2 segment between the

Table 1. AQP2 Mutations That Cause NDI

Nucleotide	Amino acid	Homozygous/ Heterozygous	Recessive/ Dominant	Reference	Functionality	Conserved (Location)	Class	Diagnosis	Treatment
64C>G	L22V	he3	r	61,97	P.(60%)	N (tmd 1)	II	13	dD
83T>C	L28P	ho	r	54		N (tmd 1)	II	1mo	dD:NR
140C>T	A47V	ho	r	54	P.(40%)	N (tmd 2)	II	?	dD-deh.:NR
170A>C	Q57P	he5	r	98	NF	N (tmd 2)	II	?	dD:NR
190G>A	G64R	ho	r	48,55,53	P.(20%)	Y (b-loop)	II	1mo	dD:NR
197-198 del	frameshift	he9	r	99			I	5 mo	?
293A>G	N68S	ho	r	56,55	NF	Y (b-loop)	II	1.5mo	e:dD-deh.:>300; dD:NR
211G>A	V71M	ho	r	54	NF	Y (b-loop)	II	?	ndD:NR
253C>T	R85*	ho	r	100			I	2mo	dD:NR
298G>A	G100R	ho	r	101			II	?	?
299C>T	G100*	ho	r	102			I	?	dD:NR
299G>T	G100V	he5	r	98	NF	N (tmd 3)	II	?	dD:NR
369delC	frameshift	ho	r	48			I	?	?
374C>T	T125M	ho, he8	r	103,104	P.(25%)	N (c-loop)	II	?	?
377C>T	T126M	ho	r	55,103,105,56	P.(20%)	N (c-loop)	II	5mo	dD-deh.:NR
439G>A	A147T	ho	r	56,55	funct.	Y (d-loop)	II	3mo	dD-deh.:NR
450T>A	D150E	he7, he10	r	106,107	NF	Y (d-loop)	II	?	?
502G>A	V168M	he4, ho	r	62,100	P.(60%)	N (tmd 5)	II	?	he:ndD:NR; ho:dD<200
523G>A	G175R	ho, he8	r	103,104,54	NF	Y (tmd 5)	II	?	?
537G>A	G180S	ho	r	101			II	?	?
543C>G	C181W	he3	r	61,108,57	NF	N (e-loop)	II	13	?
553C>G	P185A	ho	r	55	NF	Y (e-loop)	II	1wk	ndD:NR
559C>T	R187C	ho,he1	r	47,55,48	NF	Y (e-loop)	II	2wk,5mo	dD-deh.:NR
643G>T	G215C	he10	r	107		Y (tmd 6)	II	?	?
928G>A	A190T	he2	r	63,104	NF	N (e-loop)	II	?	dD:NR
587G>A	G196D	he7	r	106	NF	N (e-loop)	II	?	NR
606G>T	W202C/splice	ho	r	109		Y (e-loop)	I	4-8wk	dD:NR
c606+1G>A	splice	he6;all.2	r	54		(intr. 3)	I	2wk	dD:NR
646T>C	S216P	he1, he4	r	37,47,53,105	NF	N (tmd 6)	II	3mo	dD:NR
652delC	frameshift	he6;all.1	r	54		N (tmd 6)	I	2wk	dD:NR
721delG	frameshift	he	d	65	F	N (C-tail)	IV	12mo	ndD:NR; deh.:>300
727delG	frameshift	he	d	66	F	N (C-tail)	IV	?	dD:NR
761G>T	R254L	he	d	79	F	N (C-tail)	IV	<12mo	?
763-772del	frameshift	he	d	65	F	N (C-tail)	IV	36mo	ndD:NR
772G>A	E258K	he	d	67	F	N (C-tail)	IV	?	dD:>300
779-780insA	frameshift	he	d	64	F	N (C-tail)	IV	6-1 mo	dD:NR;>300
785C>T	P262L	he2	r	63,104	F	N (C-tail)	IV	?	dD:NR
812-818del	frameshift	he	d	65	F	N (C-tail)	IV	16mo	dD:incr.; deh.:>300
1502G>A	Transition splice site	he9	r	99				5 mo	?

Abbreviations: he, heterozygous; ho, homozygous; all, allele; r, recessive; d, dominant; NF, nonfunctional; P, partial functional (level in %); conserved yes (Y) or no (N) between AQP proteins; dD, infusion with dDAVP; ndD, nasal dDAVP; deh, dehydration; NR, nonresponsive; >300, urine more than 300 m0sm/kg water.

first and the last transmembrane domain. Because this segment forms the AQP2 water pore, the misfolding resulting from the mutations in this part of the protein illustrates the sensitivity of the pore structural changes. To establish the involvement of an identified *AQP2* gene muta-

tion in NDI and to study the underlying mechanism by which the mutation would lead to recessive NDI, the *Xenopus* oocyte expression system was mostly used. Expression of the encoded mutants in cell systems showed that almost all mutants were misfolded and, therefore, retained in the endoplasmic reticulum (ER), followed by rapid proteasomal degradation.[47,53–57] Because of this rapid degradation, AQP2 proteins could not be detected in the urine of patients suffering from recessive NDI as a result of *AQP2* gene mutations, in contrast to healthy controls.[58] The inability of these AQP2 mutants to form heterotetramers with wt-AQP2, leaving only the formation of WT-AQP2 homotetramers, provides an explanation for the healthy phenotype of the patients' parents.[13] An increased wt-AQP2 expression from the normal allele does not seem to be necessary because heterozygote AQP2 knock-out mice have a healthy phenotype and express approximately half of the AQP2 amount found in normal mice.[59] Although retained in the ER, at high expression levels that allow some of the AQP2 mutant proteins to escape from the ER and to be routed to the plasma membrane,[60] 7 mutants (L22V, A47V, G64R, T125M, T126M, A147T, and V168M) appeared to be able to confer water permeability.[54–56,61,62] Recently, the AQP2-P262L mutation was found to be involved in recessive NDI and this was rather surprising because P262 is located in the AQP2 C-terminal tail, a region that until then was believed to result in dominant NDI instead of recessive NDI. The answer to this intriguing situation came from our cell biological studies.[63] AQP2-P262L appeared to be a functional water channel that was retained in intracellular compartments different from the ER and that formed hetero-oligomers with wt-AQP2. These were all similar to AQP2 mutants in dominant NDI. Immunocytochemical analysis of cells co-expressing AQP2-P262L with wt-AQP2, however, revealed that these wt-mutant complexes were located in the apical membrane and, thus, that here, the apical sorting of wt-AQP2 was dominant over the missorting signals of AQP2-P262L. This was different from dominant NDI, because in this form the mutants retain wt-AQP2 in intracellular locations. Based on these data, the recessive inheritance of NDI in the 2 families we encountered (patients were heterozygous for an R187C or A190T mutation in one allele, combined with a P262L mutation in the other allele) can be explained as follows: in the 2 patients, AQP2-R187C and AQP2-A190T mutants are retained in the ER, and do not interact with AQP2-P262L. AQP2-P262L folds properly and assembles into homotetramers, but will be retained mainly in intracellular vesicles. The consequent lack of sufficient AQP2 proteins in the apical membrane of the patient's collecting duct cells then explains their NDI phenotype. In the parents coding for wt-AQP2 and AQP2-R187C or A190T, wt-AQP2 will not interact with either mutant but will form homotetrameric complexes, of which the insertion into the apical membrane in collecting duct cells will be regulated properly by vasopressin and will give a healthy phenotype. In the proband's healthy relatives encoding wt-AQP2 and AQP2-P262L, both proteins likely assemble into heterotetramers. However, the dominancy of wt-AQP2 sorting on the localization of AQP2-P262L will then result in a proper AVP-regulated trafficking of the heterotetrameric complexes to the apical membrane of their collecting duct cells.

AUTOSOMAL-DOMINANT NDI

At present, 7 families have been described with autosomal-dominant NDI, making it the least prominent form of NDI. The identified mutations in these families comprise deletions, insertions, and missense mutations (Table 1).

Clinical analyses of patients with recessive and dominant NDI caused by AQP2 mutations revealed 3 interesting differences.[64–67] First, in recessive NDI, symptoms (polyuria, polydipsia) are already present at birth, whereas in dominant NDI they often become apparent in the second half of the first year or later. Second, urine osmolalities of patients with recessive NDI never exceed 200 mOsm/kg H_2O, whereas in dominant NDI this can be higher. Third, some patients diagnosed with dominant NDI respond to DDAVP administration or dehydration by showing a transient increase in urine concentration. This seems to more general, because for long-QT syndrome, myotonia congenita, osteochondrodysplasia, and Rainbow syndrome/brachydactyly, it also has been ob-

served that the dominant form of the disease can be subclinically milder than the recessive form.[68–71]

Interestingly, all mutations in dominant NDI are found in the coding region of the C-terminal tail of AQP2, which is not a part of the water pore–forming segment, but, as shown by the role of phosphorylation of S256 in AQP2, has an important role in AQP2 trafficking. Indeed, all AQP2 proteins in dominant NDI appeared to be functional water channels, whereas expression in oocytes and Madin-Darby canine kidney (MDCK) cells revealed that all these AQP2 mutants in dominant NDI were indeed sorted to other subcellular locations in the cell than wt-AQP2. In polarized MDCK cells, wt-AQP2 is translocated from intracellular vesicles to the apical membrane by forskolin.[72] In contrast, the AQP2 mutant AQP2-R254L was sorted to forskolin-insensitive vesicles, AQP2-E258K was routed to the Golgi complex (oocytes) or late endosomes/lysosomes (mammalian cells), AQP2-727delG trafficked to the basolateral membrane and late endosomes/lysosomes, and AQP2-721delG, 812-818del, 763-772del, and AQP2-insA were reported to target the basolateral membrane.[63–67,73,74] Importantly, because none of these mutants were misfolded, they were able to interact with and form heterotetramers with wt-AQP2, which is in contrast to AQP2 mutants in recessive NDI.[13] In addition, in cells co-expressing wt and mutant AQP2 proteins, it appeared that the wt/mutant complexes also were missorted, which was owing to the wt-mutant interaction and dominancy of the missorting signals in the mutant proteins. Extrapolated to principal cells of the patients, this would lead to severely decreased amounts of AQP2 in the apical membrane, explaining the dominant mode of inheritance of NDI in these families.[13]

Interestingly, the *AQP2* gene mutation leading to dominant NDI can introduce a missorting signal or remove a sorting signal of wt-AQP2. With AQP2-insA, the changed C-terminal tail targeted it to the basolateral membrane, which harbored 2 basolateral sorting signals.[64] Although starting at different positions, the AQP2 mutants with the 721delG, 727delG, del763-772, or del812-818 mutations have similar extended C-terminal tails. In polarized MDCK cells, AQP2-727delG appeared to accumulate in late endosomes/lysosomes and, to some extent, in the basolateral plasma membrane,[66] whereas the other mutants were localized in the basolateral membrane.[75] Because the extended tail in AQP2-del812-818 starts only at the stop codon of wt-AQP2 and the extended tails contain the known basolateral membrane targeting di-leucine motif,[76] the missorting of these mutants was suggested to be owing to this introduced basolateral sorting signal.

Early studies in oocytes suggested that the exchange of E258 for a lysine (E258K) also introduced a missorting signal because deletion of the segment surrounding E258 greatly restored the plasma membrane expression of the AQP2-E258K mutant.[67] This was corroborated by the study of Procino et al,[73] who found that the mutation precluded phosphorylation of AQP2-E258K at S256 by Golgi casein kinase, and suggested that this resulted in its missorting to multivesicular bodies. By analysis of several mutants in polarized MDCK cells, however, we recently discovered that AQP2-E258K is well phosphorylated at S256 and that mimicking phosphorylation at S256 in AQP2-E258K by changing S256 into an aspartate did not change its localization to multivesicular bodies.[77] Moreover, because AQP2-E258R and AQP2-E258A also were sorted to multivesicular bodies, we concluded that it was the loss of E258 in AQP2 instead of introduced signal that caused the missorting of AQP2-E258K. Because wt-AQP2 also is sorted to multivesicular bodies when constitutively coupled to a ubiquitin moiety,[78] it will be interesting to investigate whether AQP2-E258K in some way structurally mimics ubiquitinated wt-AQP2. Besides AQP2-E258K, AQP2-R254L also is missorted because of a lost signal because this mutation destroyed the protein kinase A consensus site, resulting in the lack of forskolin-induced phosphorylation of AQP2-R254L at S256.[63] Correction of its S256 phosphorylation by substituting S256 for an aspartate, however, resulted in the expression of the corresponding protein (AQP2-R254L-S256D) in the apical and basolateral membrane,

indicating that even if phosphorylation of this mutant could be overcome, the leucine at position 256 would induce a partial missorting to the basolateral membrane.

MOUSE MODELS TO STUDY CONGENITAL NDI

Recently, 5 congenital NDI mouse models were created,[79–83] providing novel and important in vivo information on the role of AQP2 in water conservation in health and disease. Rojek et al[80] generated mice lacking functional AQP2 either completely (AQP2-total-knock-out [KO]) or only in the collecting ducts (AQP2-CD-KO) by exploiting the Cre/loxP technology. LoxP sites were inserted into *AQP2* introns 2 and 3, and transgenic mice were bred with strains expressing Cre recombinase under the control of CD-specific Hoxb7- or global EIIa promoter. Consistent with the complete lack of functional AQP2 in the homozygous knock-in of an AQP2 mutant in recessive NDI, AQP2-T126M,[59] AQP2-total-KO mice looked healthy at birth but failed to thrive and died within 2 weeks as a result of severe urine concentrating defects and massive contraction of extracellular fluid volume. Moreover, hydronephrosis also was observed. A nearly 100% absence of AQP2 in the CDs was observed in the AQP2-CD-KO mice, but clear expression of AQP2 in the connecting tubules (CNTs) was visible. Although these mice survived to adulthood, growth retardation and progressive hydronephrosis was observed, which was caused by a 10-fold increased urine volume compared with controls. Consistently, their urine osmolality was 10-fold decreased. The other 4 congenital NDI mice[79–83] showed similar phenotypes, and increased serum blood urea nitrogen levels were observed in the AQP2-S256L mice and in mice with the distal C-tail deleted,[82] indicating renal failure. Although growth retardation often is observed in uncontrolled NDI in human beings, hydronephrosis and renal failure rarely have been noted[84] and this indicates that NDI is more severe in mice.

The first mouse model used to study dominant NDI was generated by Sohara et al[82] and this was performed by homologous recombination of part of the last exon of the human AQP2 mutant (763-772del). The AQP2-763-772del heterozygous mice survived, in line with the observation that the age of onset of dominant NDI in human beings often is later and subclinically milder compared with recessive NDI. Although the urine concentrating ability was reduced severely compared with wild-type mice, administration of DDAVP did significantly increase urine concentration in the heterozygous mice. Although an increased urine osmolality with DDAVP also was observed in some human patients suffering from dominant NDI, this is not found commonly. Treatment of these mice with rolipram (a phosphodiesterase [PDE]-4 inhibitor), but not PDE3 or PDE5 inhibitors, resulted in an increased urine concentration. If reproducible in human beings, this suggests that PDE4 inhibitors might be an interesting therapy to treat dominant NDI.

Although the molecular mechanism causing autosomal-recessive and -dominant NDI in vivo appeared similar to the in vitro studies,[85] the relation between the location of the AQP2 mutation and the trait of inheritance in mice came with some surprises. Although AQP2-S256L interacted with wt-AQP2 and was impaired in its phosphorylation at S256, similar to AQP2-R254L, it caused recessive instead of dominant NDI in mice.[82] The considerable apical membrane expression of AQP2 in the heterozygous mice explained this difference. Assuming that the expression of wt-AQP2 and AQP2-S256L in these mice is similar, the differences in inheritance suggest that AQP2-R254L and AQP2-S256L differ in their ability to cause missorting of wt-AQP2 (ie, AQP2-R254L stronger than AQP2-S256L). This is in line with our in vitro data that AQP2-P262L, which also interacts with wt-AQP2, causes recessive instead of dominant NDI, because its missorting is overruled by wt-AQP2 when co-expressed.[63]

A greater surprise was found by Lloyd et al,[81] who investigated mice suffering from NDI as a result of an F204V AQP2 mutation. Similar to mutants in recessive NDI, this mutation is localized between the first and sixth transmembrane domain of AQP2 and also resulted in an ER-retained protein. However, although AQP2 null mutants and a mouse model homozygous for a human AQP2 mutant causing recessive NDI (AQP2 -T126M) die soon after birth because of dehydration, mice homozygous for AQP2-

F204V were viable because sufficient amounts of this mutant appeared at the apical membrane of principal cells to guarantee survival. Moreover, in vitro and in heterozygote mice, AQP2-F204V clearly interacted with wt-AQP2, revealing that an AQP2 mutant with a mutation between the first and sixth transmembrane domain may interact with wt-AQP2. Interestingly, a similar situation might be the case for AQP2-V168M because this mutant was less ER-retained when expressed in cells, and the phenotype of human beings homozygous for this mutation varied from severe NDI to no NDI at all. Whether this or some other AQP2 mutants in human recessive NDI may indeed interact with wt-AQP2, however, remains to be established.

THERAPIES IN CONGENITAL NDI

The most important component of treatment of NDI, whether acquired or congenital, is replacement of urinary water losses by an adequate supply of fluid, in combination with a decreased solute diet to decrease obligatory water excretion. Most infants with NDI, however, cannot drink the required amounts of fluid. Initially, a diet low in sodium (1 mmol/kg/d) as well as protein (2 g/kg/d) was recommended, but severe limitations of dietary protein may introduce serious nutritional deficiencies and therefore a dietary restriction of sodium only is preferable. Nowadays, the treatment usually consists of diuretics such as hydrochlorothiazide and amiloride, which have been shown to be effective in lowering urine volume in NDI.[86,87] Administration of hydrochlorothiazide combined with either a COX inhibitor such as indomethacin or the potassium-sparing diuretic amiloride was shown to be more effective in reducing urine volume than the thiazide diuretic alone.[43,88–91] However, long-term use of prostaglandin-synthesis inhibitors often is complicated by hematopoietic and gastrointestinal side effects. Renal dysfunction also can occur during indomethacin treatment, mostly consisting of a reduction in the glomerular filtration rate. Although these treatments decrease excessive water excretion to a great extent, patients with this therapy still can void 8 to 10 L/d.

During the past 10 years, different approaches to develop alternative therapies for NDI have been tested in vitro. One of the most promising approaches at the moment is the use of cell-permeable V2R or V1R inverse agonists in the rescue of ER-retained, but functional, V2R mutants in NDI (described by Dr. Daniel Bichet on page 245 in this issue).[92–94] In the coming years it will be exciting to see how well these compounds work out in vivo. Because most AQP2 mutants in NDI are ER-retained, and many of them are functional, the rescue approach as described for V2R mutants earlier also would be very useful for AQP2 mutants. For AQP water channels, however, no specific interacting compounds are known. Although the number of patients with recessive NDI as a result of AQP2 mutations is low and the conventional therapy relieves NDI to a great extent, identification of compounds that can rescue AQP2 mutants in NDI often is considered not worth the effort. However, because of its role in excessive water reabsorption in congestive heart failure, cirrhosis, SIADH and pre-eclampsia, which are encountered quite frequently, efforts are on their way to identify AQP2-specific inhibitors. Possibly, cell permeable forms of these compounds might become the drugs with which to rescue AQP2 mutants in NDI and to provide an additional therapy for many of the NDI patients with *AQP2* gene mutations.[96]

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