Congenital nephrogenic diabetes insipidus (NDI) is a rare disorder of the kidney characterized by the failure to concentrate urine despite normal or elevated plasma concentrations of the antidiuretic hormone arginine vasopressin (AVP). The identification, characterization, and mutational analysis of two different genes, i.e., the AVP receptor 2 gene (AVPR2) and the vasopressin-sensitive water channel gene (aquaporin-2 [AQP2]), provide the basis for our understanding of two different hereditary forms of NDI: X-linked NDI and autosomal recessive NDI. A majority (>90%) of congenital NDI patients have AVPR2 mutations: Of 115 families with congenital NDI that were referred to our laboratories in Montreal and Berlin, 105 families had AVPR2 mutations and 10 had AQP2 mutations. When studied in vitro, most AVPR2 mutations lead to receptors that are trapped intracellularly and are unable to reach the plasma membrane. A minority of the mutant receptors reach the cell surface but are unable to bind AVP or to properly trigger an intracellular cAMP signal. Similarly, AQP2 mutant proteins are misrouted and cannot be expressed at the luminal membrane. The advances described in this review provide diagnostic tools for physicians caring for these patients and open the door for the development of therapeutic strategies based on gene transfer.

Cellular Actions of Vasopressin and Molecular Biology of NDI

The conservation of water by the human kidney is a function of the complex architecture of renal tubules within the renal medulla. The principal cells of the renal collecting tubules are responsive to the neurohypophyseal antidiuretic hormone AVP. The major action of AVP is to facilitate urinary concentration by allowing water to be transported passively down an osmotic gradient between the tubular fluid and the surrounding interstitium. The process of this counter multiplication system and the action of AVP on principal collecting duct cells are represented in Figure 1.

The first step in the antidiuretic action of AVP is its binding to the vasopressin V2 receptor (AVPR2 in Figure 1; a three-dimensional model of the vasopressin V2 receptor where AVP is docked is shown in Figure 2A) located on the basolateral membrane of collecting duct cells. This step initiates a cascade of events—receptor-linked activation of G-protein (Gαi), activation of adenylyl cyclase, production of cAMP, and stimulation of protein kinase A—that leads to the final step in the antidiuretic action of AVP, i.e., the exocytic insertion of specific water channels, AQP2, into the luminal membrane, thereby increasing the water permeability of that membrane. These water channels are members of a superfamly of integral membrane proteins that facilitate water transport.

In congenital NDI, the renal collecting ducts are resistant to the antidiuretic action of AVP or to its antidiuretic analog 1-desamino-8-D-arginine vasopressin. This is a rare, but now well described, entity secondary either to mutations in the AVPR2 gene (X-linked NDI [Online Mendelian Inheritance in Man (OMIM), Johns Hopkins University, Baltimore, MD; MIM No.: 304800]), which codes for the antidiuretic (V2) receptor, or to mutations in the AQP2 gene (autosomal recessive NDI [OMIM, Johns Hopkins University; MIM No.: 222000]), which codes for the vasopressin-dependent water channel (9–11). Of 98 families with congenital NDI referred to our laboratory in Montreal, 90 families have AVPR2 mutations and eight have AQP2 mutations. Most of the affected patients (with AVPR2 or AQP2 mutations) have a full phenotype characterized by the inability to increase the urinary osmolality value above the plasma osmolality value during a pharmacological infusion of 1-desamino-8-D-arginine vasopressin (7). Only three AVPR2 mutations are characterized...
Figure 1. Schematic representation of the nephron with selected areas involved in the urinary concentrating mechanism. (Top Left Panel) The reabsorption of NaCl, but not water, from the ascending limbs of Henle, initiate countercurrent multiplication within the renal medulla. The Na-K-2Cl cotransporter (inhibited by bumetanide) in the medullary thick ascending limb is shown. (Right Panel) Plasma arginine vasopressin (AVP) increases water permeability in collecting ducts: The hormone is bound to the vasopressin V2 receptor (a G-protein linked receptor) on the basolateral membrane. AVP activates adenylyl cyclase, increasing the intracellular concentration of cAMP. The topology of adenylyl cyclase is characterized by two tandem repeats of six hydrophobic putative transmembrane domains separated by a large cytoplasmic loop and terminating in a large intracellular tail. Generation of cAMP follows receptor-linked activation of the heterometric G-protein (G) and interaction of the free Gαi-chain with the adenylyl cyclase catalytic subunit. A cAMP-dependent protein kinase A (PKA) is the target of the generated cAMP. Cytoplasmic vesicles carrying the water channel proteins (represented as homotetrameric complexes) are fused to the luminal membrane in response to vasopressin, thereby increasing the water permeability of this membrane. When vasopressin is not available, water channels are retrieved by an endocytic process, and water permeability returns to its original low rate. Aquaporin-3 (AQP3) and AQP4 are expressed on the basolateral membrane. Vasopressin also increases the permeability of the terminal part of the collecting duct to urea, resulting in movement of urea into the medullary interstitium. This process is provided by urea transporters (represented here with 10 transmembrane domains). Both the inner medullary collecting duct and thin descending limbs of short loops of Henle express a urea transporter (reprinted from reference 2 with permission).

by a mild phenotype with a less severe clinical disease and the possibility of increasing urinary osmolality to approximately 400 mosmol/L during a dehydration test with a concomitant plasma sodium concentration less than 150 mEq/L (12,13).

The human V2 receptor gene AVPR2 is located in chromosome region Xq28 and has three exons and two small introns (14,15). The sequence of the cDNA predicts a polypeptide of 371 amino acids with a structure typical of guanine-nucleotide (G) protein-coupled receptors with seven transmembrane, four extracellular, and four cytoplasmic domains (16) (Figure 2A).

The human AQP2 gene is located in chromosome region 12q13 and has four exons and three introns (10,11). It is predicted to code for a polypeptide of 271 amino acids that is organized into two repeats oriented at 180° to each other and that has six membrane-spanning domains, with both terminal ends located intracellularly, and conserved asparagine-proline-
Figure 2. (A) Ribbon representation of the AVP receptor 2 (AVPR2). This is a side view from a direction parallel to the cell membrane surface. The positioning of transmembrane domains 1 through 7 is counterclockwise when viewed from the extracellular surface of the receptor. The transmembrane helices are shown in red, and the intracellular and extracellular loops are shown in purple. The model is oriented such that the extracellular side is at the top of the image. This hypothetical model of interaction between AVP and its V₂ receptor is constructed according to the model published by Mouillac et al. (3) pertaining to the V₁₅ receptor. AVP is supposed to be completely embedded into a 15 to 20 Å cleft defined by the transmembrane helices 2 through 7 of the receptor. The localization of the R 137 residue is represented. This image was produced using the MidasPlus program (Computer Graphics Laboratory, University of California, San Francisco, CA; supported by National Institutes of Health Grant RR-01081). (B) Expression of the R137H mutant in L cells. The R137H mutant had unaltered affinity for tritiated AVP but failed to stimulate adenylate cyclase (28). Inset shows the localization of the missense amino acid in a planar representation. Only the first three transmembrane domains are represented.

Clinical Characteristics of AVPR2 Mutations, Incidence, Population Genetics, Ancestral Mutation and de novo Mutations, and Mechanisms of AVPR2 Mutations

The AVPR2 gene is located in chromosome region Xq28 and, as a result, males who have an AVPR2 mutation have a phenotype characterized by early dehydration episodes, hypernatremia, and hyperthermia as early as the first week of life. The dehydration episodes can be so severe that they lower arterial blood perfusion pressure to a degree insufficient to sustain adequate oxygenation to the brain, kidneys, and other organs. Mental and physical retardation and renal failure are the classical “historical” consequences of a late diagnosis and lack of treatment. Heterozygous females exhibit variable degrees of polyuria and polydipsia because of skewed X chromosome inactivation. The onset and severity of the clinical manifestations of autosomal recessive NDI are similar to those of X-linked NDI.
Figure 3. Schematic representation of the AQP2 protein and identification of 10 AQP2 mutations. A monomer is represented with six stretches of hydrophobic sequences that are suggestive of six transmembrane helices. The major intrinsic proteins of lens (see text) share an NPA (Asn-Pro-Ala) motif in each of the two prominent loops. AQP1 (and by analogy AQP2) is a homotretamer containing four independent aqueous channels.

The early symptomatology of the nephrogenic disorder and its severity in infancy is clearly described by Crawford and Bode (17). The first manifestations of the disease could be recognized during the first week of life. The infants are irritable, cry almost constantly, and, although eager to suck, will vomit milk soon after ingestion unless prefed with water. The history given by the mothers often includes persistent constipation; erratic, unexplained fever; and failure to gain weight. Even though the patients characteristically show no visible evidence of perspiration, increased water loss during fever or in warm weather exaggerates the symptoms.

Unless the condition is recognized early, children will experience frequent bouts of hypertonic dehydration, sometimes complicated by convulsion or death. Mental retardation is a frequent consequence of these episodes. The intake of large quantities of water, combined with the patient’s voluntary restriction of dietary salt and protein intake, leads to hypocaloric dwarfism beginning in infancy. Affected children frequently develop lower urinary tract dilatation and obstruction, probably secondary to the large volume of urine produced (18). Dilatation of the lower urinary tract is also seen in primary polydipsic patients and in patients with central (neurogenic) diabetes insipidus (19,20). Chronic renal insufficiency may occur by the end of the first decade of life and could be the result of episodes of dehydration with thrombosis of the glomerular tufts (17).

Generally, X-linked NDI is a rare disease with an estimated prevalence of approximately four per 1 million males and a carrier frequency of $7.4 \times 10^{-6}$; these figures are based on the number of patients with X-linked NDI known in Quebec (21). In defined regions of North America, however, the prevalence is much higher. It is assumed that the patients in these regions are progeny of common ancestors. An example is the Mormon pedigree, with its members residing in Utah (Utah families); this pedigree was originally described by Cannon (22,23). The “Utah mutation” is a missense mutation (L312X) predictive of a receptor that lacks transmembrane domain 7 and the intracellular C terminus (23). The largest known kindred with X-linked NDI is the Hopewell family, named after the Irish ship Hopewell that arrived in Halifax, Nova Scotia, in 1761 (24). Aboard the ship were members of the Ulster Scot clan, descendants of Scottish Presbyterians who migrated to the Ulster Province of Ireland in the 17th century and left Ireland for the new world in the 18th century.
Whereas families arriving with the first emigration wave settled in northern Massachusetts in 1718, the members of a second emigration wave, passengers of the Hopewell, settled in Colchester County, Nova Scotia. According to the “Hopewell hypothesis” (24), most NDI patients in North America are progeny of female carriers from the second emigration wave. This assumption is based mainly on the high prevalence of NDI among descendants of the Ulster Scots residing in Nova Scotia. In two villages with 2500 inhabitants, 30 patients have been diagnosed, and the carrier frequency has been estimated at 6% (21). Given the numerous mutations found in North American X-linked NDI families, the Hopewell hypothesis cannot be upheld in its originally proposed form. However, among X-linked NDI patients in North America, the W71X mutation is more common than another AVPR2 mutation. It is a null mutation (W71X; 23,25) (Figure 4) predictive of an extremely truncated receptor consisting of the extracellular N terminus, the first transmembrane domain, and the N-terminal half of the first intracellular loop. Because the original carrier cannot be identified (21), it is not clear whether the Hopewell mutation was brought to North America by Hopewell passengers or by other Ulster Scot immigrants. Seventy-two different putative disease-causing mutations in the AVPR2 gene have now been reported in 102 unrelated families with X-linked NDI (Figure 4). The diversity of AVPR2 mutations found in many ethnic groups (Caucasians, Japanese, African-Americans, Africans) and the rareness of the disease are consistent with an X-linked recessive disease that in the past was lethal for male patients and was balanced by recurrent mutations. In X-linked NDI, loss of mutant alleles from the population occurs because of the higher mortality of affected males compared with healthy males, whereas gain of mutant alleles occurs by mutation. If affected males with a rare X-linked recessive disease do not reproduce and if mutation rates are equal in mothers and

![Figure 4. Schematic representation of the V2 receptor and identification of 72 AVPR2 mutations, which include 36 missense, 10 non-sense, 18 frameshift, two inframe deletions, one splice-site, and five large deletion mutations. The four large deletions are characterized incompletely and are not included in the figure. Predicted amino acids are given as the one-letter code. Solid symbols indicate the predicted location of the mutations; an asterisk indicates two different mutations in the same codon. The names of the mutations were assigned according to the conventional nomenclature. The extracellular (E to E\text{IV}) cytoplasmic (C\text{I} to C\text{IV}), and transmembrane domains (TM\text{I} to TM\text{VIII}) are labeled from the N terminus to the C terminus, according to Sharif and Hanley (40). E\text{I}: 98del28, 98ins28, 113delCT. TM\text{I}: L44F, L44P, L53R, L62P. TM\text{II}: 85delCT, 253del35, 255del9. C\text{I}: 274insG, W71X. TM\text{III}: H80R, L83P, D85N, V88M, 337delCT, P95L. E\text{IV}: R106C, 402delCT, C112R, R113W. TM\text{IV}: Q119X, Y124X, S126F, Y128S, A132D. C\text{IV}: R137H, R143P, 528delE7, 528delG. TM\text{V}: W164S, S167L, S167T. E\text{V}: R181C, G185C, R202C, T204N, 684delTA, Y205C, V206D. TM\text{VI}: L219R, Q225X, 753insC. C\text{V}: E231X, 763delA, 786delG, 824delX, 804insG, 804delG, 834delA, 855delG. TM\text{VII}: V277A, V278, W280C, W284X, A285P, P286L, L292P, W293X. E\text{VII}: 977delG, 982-2A→G. TM\text{VIII}: L312X, P322H, S322S, W323R. C\text{VIII}: R337X. Whereas deletions and insertions can be attributed to slipped mispairing during DNA replication favored by direct repeats, complementary repeats and symmetric sequences in the vicinity of the mutation (more than 50% of the single-base substitutions) can be explained by mutations in CpG dinucleotides, which are hot spots for genetic disease and which are relatively common in the human V2 receptor gene (26).]
AQP2 Mutations

The AQP2 gene is located in chromosome region 12q13. Males and females affected with congenital NDI have been described who are homozygous for a mutation in the AQP2 gene or who carry two different mutations (9,10,12,30,31) (Figure 3). Functional expression studies showed that Xenopus oocytes injected with mutant cRNA had abnormal coefficient of water permeability, whereas Xenopus oocytes injected with both normal and mutant cRNA had coefficient of water permeability similar to that of normal constructs alone. These findings provide conclusive evidence that NDI can be caused by homozygosity for mutations in the AQP2 gene. More recently, we obtained evidence in three ancestrally independent families to suggest that both autosomal dominant and autosomal recessive NDI phenotypes could be secondary to novel mutations in the AQP2 gene. Reminiscent of expression studies done with AVPR2 proteins, Deen and coworkers also demonstrated that the major cause underlying autosomal recessive NDI is the misrouting of AQP2 mutant proteins (31,32).

Carrier, Perinatal Testing, and Perspectives

Over the past few years, it has become clear that congenital NDI is caused by an inactivating mutation of a G-protein-coupled receptor (V2 receptor) or a water channel (AQP2). The time of onset of the disease (shortly after birth) and the clinical symptoms do not differ between the two forms. However, the two forms can be distinguished by clinical testing: Whereas desmopressin elicits extrarenal (coagulation and vasodilatory) responses in patients with autosomal recessive NDI, patients with X-linked NDI lack extrarenal response to desmopressin (9,33,34).

Identification of the molecular defects underlying congenital NDI is of immediate clinical significance, allowing diagnosis by gene analysis. We encourage physicians who follow families with X-linked and non-X-linked NDI to recommend molecular genetic analysis, because early diagnosis and treatment of affected infants can avert the physical and mental retardation associated with episodes of dehydration. Diagnosis of X-linked NDI was accomplished by mutation testing of a sample of cord blood in five of our patients. These patients were immediately treated with abundant water intake, a low-sodium diet, and hydrochlorothiazide. They never experienced episodes of dehydration, and their physical and mental development is normal. Gene analysis should be performed in newborns with a family history of NDI and in patients of all age groups with a firm diagnosis of congenital NDI, with or without a family history. It may also be considered in babies presenting with continuing fever of unknown origin, vomiting, constantly low urine osmolality, and failure to thrive. Gene analysis is also important for the identification of nonobligatory female carriers in families with X-linked NDI. Most females heterozygous for a mutation in the V2 receptor do not present with clinical symptoms; few are severely affected (35,36) (D.G. Bichet, unpublished observations).

All complications of congenital NDI are prevented by an adequate water intake. Thus, patients should be provided with unrestricted amounts of water from birth to ensure normal development. In addition to a low-sodium diet, the use of diuretics (thiazides) or indomethacin may reduce urinary output. This advantageous effect has to be weighed against the side effects of these drugs (thiazides: electrolyte disturbances; indomethacin: reduction of glomerular filtration rate and gastrointestinal symptoms). With the identification of the genes responsible for congenital NDI, a causative treatment based on gene transfer has become possible.

Two prerequisites crucial for gene therapy seem to be fulfilled in both forms of congenital NDI. (1) The defect in the kidney appears to be restricted to water reabsorption with no other functional or histological defects. Unlike central diabetes insipidus (37), retinitis pigmentosa (see above), and many other diseases, a deterioration of kidney function due to progressive structural changes is not observed. Thus, organ integrity seems to be preserved. (2) Recent experiments with rats show that adenoviral-mediated gene transfer to the tubular system of the kidney can be achieved either by selective perfusion of the renal artery or by retrograde infusion through a catheter placed into the pelvic cavity (38). Depending on the route, expression of the reporter gene (β-galactosidase) is observed in proximal tubule cells (kidney perfusion via renal artery) or tubular cells of the papilla and medulla (retrograde infusion).

The mutations in the V2 receptor associated with X-linked NDI were the first naturally occurring mutations found in the very large group of G-protein-coupled hormone receptors. Within the past few years, however, a number of diseases were...
shown to be caused by mutations in genes encoding G-protein-coupled receptors. In addition to retinitis pigmentosa and X-linked NDI, examples of such diseases/symptoms include: stationary night blindness, color blindness/alteration color perception, primary adrenocortical deficiency, hypocalciuric hypocalcemia/hyperparathyroidism, hypercalcemia/metaphyseal chondrodysplasia, hypocalcemia, male precocious puberty, expression of mutant receptors on the cell surface. Here, the main defect of many inactivating mutations is the reduced biological activity of the individual protein. At present, little is known about the cellular routing of G-protein-coupled receptors. Progress in this field will be crucial for the understanding of the clinical phenotypes of receptor diseases on a molecular level and for the development of therapeutic strategies based on gene transfer.

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