Congenital Nephrogenic Diabetes Insipidus

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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 type 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 (1). 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 (G), activation of adenyl 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 superfamily of integral membrane proteins that facilitate water transport (4,5). Aquaporin-1 (AQP1; also known as CHIP, channel-forming integral membrane protein of 28 kD) was the first protein shown to function as a molecular water channel and is constitutively expressed in mammalian red cells, renal proximal tubules, thin descending limbs, and other water-permeable epithelia (6). At the subcellular level, AQP1 is localized in both apical and basolateral plasma membranes that may represent entrance and exit routes for transepithelial water transport. In contrast to AQP2, limited amounts of AQP1 are localized in membranes of vesicles or vacuoles. In the basolateral membranes, AQP1 is localized to both basal and lateral infoldings. AQP2 is the vasopressin-regulated water channel in renal collecting ducts. It is exclusively present in principal cells and inner medullary collecting duct cells and is diffusely distributed in the cytoplasm in the euhydrated condition, whereas apical staining of AQP2 is intensified in the dehydrated condition or after vasopressin administration. These observations are thought to represent the exocytic insertion of preformed water channels from intracellular vesicles into the apical plasma membrane (the shuttle hypothesis) (Figure 1). AQP3 and AQP4 are the water channels in basolateral membranes of renal medullary collecting ducts.

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 (7,8). 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...
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-
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

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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 (E₁ to E₁) and intracellular (C₁ to C₁) domains and the transmembrane domains (T₁ to T₁) are labeled from the N terminus to the C terminus, according to Sharif and Hanley (40). E₁: 98del28, 98ins28, 113delCT. T₁: L44F, L44P, L53R, L62P. T₁ and C₁: 253del35, 255del9. C₁: 274insG, W71X. T₁: H80R, H83P, D85N, V88M, 337delCT, P95L. E₁: R106C, 402delCT, C112R, L133W. T₁: Q119X, Y124X, S126F, Y128S, A132D. C₁: R137H, R143P, 528delT, 528delG. T₁: W164S, S167L, S167T. E₁: R181C, G185C, R202C, T204N, 684delTA, Y205C, V206D. T₁: L219R, Q225X, 753insC. C₁: E231X, 763delA, 786delG, 824delX, 804insG, 804delG, 834delA, 855delG. T₁: V277A, V278, Y280C, W284X, A285P, P286L, P286R, L292P, W293X. E₁: 977delG, 982→A→G. T₁: S312X, P322H, P322S, W323R. C₁: 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).
fathers, then, at genetic equilibrium, one-third of new cases of affected males will be due to new mutations. We and others have described ancestral mutations, de novo mutations, and potential mechanisms of mutagenesis (26). These data are reminiscent of those obtained from patients with late-onset autosomal-dominant retinitis pigmentosa. In one-fourth of these patients, the disease is caused by mutations in the light receptor rhodopsin. Here, too, many different mutations (approximately 100), spread throughout the coding region of the rhodopsin gene, have been found (27).

The basis of loss of function or dysregulation of 28 different mutant V₂ receptors (including non-sense, frameshift, deletion, or missense mutations) has been studied using in vitro expression systems (Figure 2B). Most of the mutant V₂ receptors tested were not transported to the cell membrane and were thus retained within the intracellular compartment (8). Schöneberg and coworkers (29) pharmacologically rescued truncated V₂ receptors by coexpression of a polypeptide consisting of the last 130 amino acids of the V₂ receptor. Four of the six truncated receptors (E242X, 804delG, 834delA, and W284X) regained considerable functional activity as demonstrated by an increase in the number of binding sites and stimulation of adenylate cyclase activity. These in vitro results are potentially promising avenues for the gene therapy of AVPR2 mutations.

**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/altred color perception, primary adrenocortical deficiency, hypocalciuric hypercalcemia/hyperparathyroidism, hypocalcemia/metaphyseal chondrodysplasia, hypocalcemia, male precocious puberty, male pseudohermaphroditism, hyperfunctioning thyroid adenoma, and Hirschsprung’s disease (reviewed in reference 39). The main defect of many inactivating mutations is the reduced expression of mutant receptors on the cell surface. Here, the loss of receptor function occurs regardless of the remaining 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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