SEMINARS IN
NEPHROLOGY

Hereditary Polyuric Disorders: New Concepts and Differential Diagnosis

Daniel G. Bichet

The identification, characterization, and mutational analysis of genes coding for key proteins to the mechanisms of urine concentration provide the basis for understanding the 2 types of hereditary nephrogenic diabetes insipidus (NDI): a pure type characterized by loss of water only, and a complex type characterized by loss of water and ions. Patients with hereditary NDI bearing mutations in *AVPR2*, the gene coding for the arginine vasopressin 2 receptor, or in *AQP2*, the gene coding for the vasopressin-sensitive water channel, have a pure NDI phenotype with loss of water, but normal conservation of sodium, potassium, chloride, and calcium. Patients bearing inactivating mutations in 1 of the 5 genes (*SLC12A1*, *KCNJ1*, *CLCNKB*, *CLCNKA*, and *CLCNKB* in combination, or *BSND*) that encode the membrane proteins of the thick ascending limb of the loop of Henle have a complex polyuro-polydipsic syndrome with loss of water, sodium, chloride, calcium, magnesium, and potassium. The purpose of this article is to increase the general awareness of these congenital NDI patients to prevent severe episodes of dehydration and provide precise molecular diagnosis and treatment.

KEYWORDS nephrogenic diabetes insipidus, urine concentration, *AVPR2* mutations, *AQP2* mutations, Bartter syndrome, hereditary salt-losing tubulopathy, mutational analysis, genetic testing

Two sisters (9 and 11 years old) were referred for investigation of congenital polyuria and repeated episodes of dehydration during their first year of life. Both were born prematurely (32 and 29 weeks, respectively) of apparently nonconsanguineous French-Canadian parents and were transferred in the perinatal period from the Lac St-Jean region in Northern Quebec to a pediatric hospital in Montreal for rehydration.

The prematurity was attributed to polyhydramnios, which occurred during both pregnancies.

The urine osmolalities of these 2 patients were unresponsive to vasopressin. A diagnosis of congenital partial nephrogenic diabetes insipidus (NDI) was made and the polyuro-polydipsic manifestations subsequently were reasonably well controlled with hydrochlorothiazide and indomethacin. However, hyponatremic dehydration episodes were encountered often. A renal ultrasound showed medullary nephrocalcinosis in both patients.

A dehydration test (Fig 1) performed 5 years before the present referral showed, in both patients, a plasma sodium level of 143 to 145 mEq/L, with a concomitant urine osmolality of 231 to 253 mOsm/kg and plasma vasopressin concentrations of 8 to 32 pg/mL. Urine osmolality was unchanged or decreased after the administration of 1.0 μg intravenous 1-desamino[8-D-arginine]vasopressin (dDAVP).

On physical examination, both girls were small for their age (10th percentile) and were concerned and reluctant about the possibility of repeating another dehydration test. Blood pressure, pulse, and oral temperature readings were normal. A dDAVP infusion test was performed as previously described.[1,2] Indomethacin treatment was discontinued 1 week before testing and water was not restricted during the test. The dDAVP test showed normal plasma sodium values but consistently low plasma potassium concentrations and a relatively mild to moderate concentration defect with urine osmolalities greater than 250 mOsm/kg after dDAVP (Fig 2).

Groupe d'Étude des Protéines Membranaires; and the Université de Montréal, Research Centre and Nephrology Service, Hôpital du Sacré-Coeur de Montréal, Montréal, Québec, Canada.
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Address reprint requests to Dr. Daniel G. Bichet, Research Centre, Hôpital du Sacré-Coeur de Montréal, 5400 Boulevard Gouin West, Montréal, Québec, H4J 1C5 Canada. E-mail: Daniel.bichet@umontreal.ca

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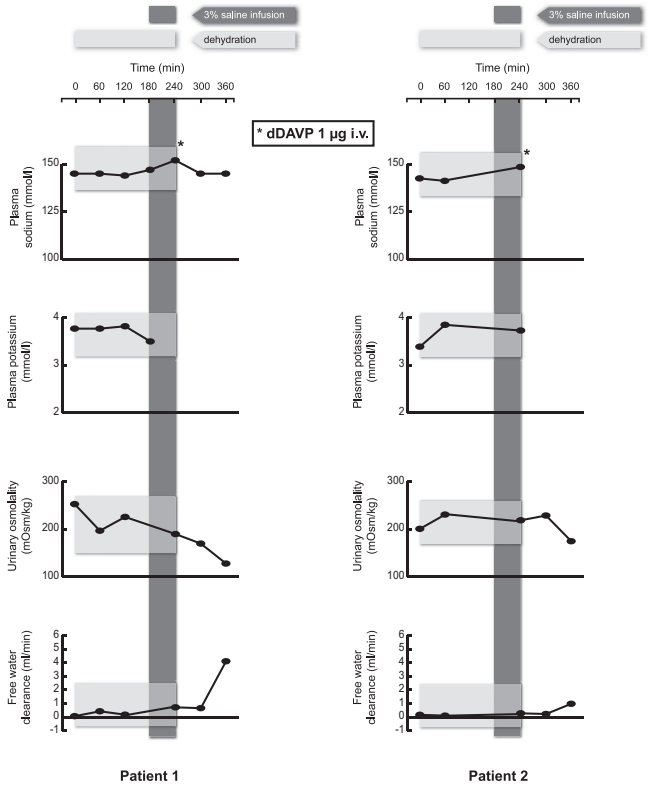

Figure 1 Measurements during a dehydration test followed by the administration of 1 μg of dDAVP intravenously to 2 sisters subsequently found to have Bartter syndrome (see Fig 2). The dehydration test was performed at 4 (patient 1, left) and 6 years of age (patient 2, right). The 24-hour urine volume was 90 mL/kg of body weight in patient 2 (normal ≤30 mL/kg body weight). Plasma arginine vasopressin (AVP and plasma renin activity were very high during the test (21-51 pg/mL for AVP and 19-23 ng/mL/h for plasma renin activity, respectively).

Regulated Transepithelial Water Permeability in the Collecting Duct: Role of AVPR2 and AQP2

The urinary concentration mechanisms require near-isosmolar fluid absorption in the proximal tubule, countercurrent multiplication/exchange to generate a hypertonic medullary interstitium, and regulated transepithelial permeability in the collecting duct.[3] The basolateral AVPR2 and the luminal AQP2 proteins are 2 critical components of the transepithelial water permeability of the principal cells of the collecting ducts (Fig 3) because loss-of-function of either of these proteins will result in NDI.

The human gene that codes for the V_2 receptor (*AVPR2*) is located in chromosome region Xq28 and has 3 exons and 2 small introns. The sequence of the complementary DNA predicts a polypeptide of 371 amino acids with 7 transmembrane, 4 extracellular, and 4 cytoplasmic domains (Fig 4). The V_2 receptor is 1 of 701 members of the rhodopsin family within the superfamily of guanine-nucleotide (G) protein–coupled receptors[4] (see also the perspective by Perez[5]). The activation of the V_2 receptor on renal collecting tubules stimulates adenylyl cyclase via the stimulatory G protein and promotes the cyclic adenosine monophosphate (cAMP)-mediated incorporation of water pores into the luminal surface of these cells. This process is the molecular basis of the vasopressin-induced increase in the osmotic water permeability of the apical membrane of the collecting tubule.[6]

The gene that codes for the water channel of the apical membrane of the kidney collecting tubule has been designated *aquaporin 2* (*AQP2*) and was cloned by homology to the rat aquaporin of the collecting duct. The human *AQP2* gene is located in chromosome region 12q13 and has 4 exons and 3 introns. It is predicted to code for a polypeptide of 271 amino acids that is organized into 2 repeats oriented at 180° to each other and has 6 membrane-spanning domains, with both terminal ends located intracellularly, and conserved asparagine-proline-alanine boxes (Fig 5). It is now well recognized that aquaporins are transmembrane channels that are found in cell membranes of all life forms that efficiently transport water while excluding pro-

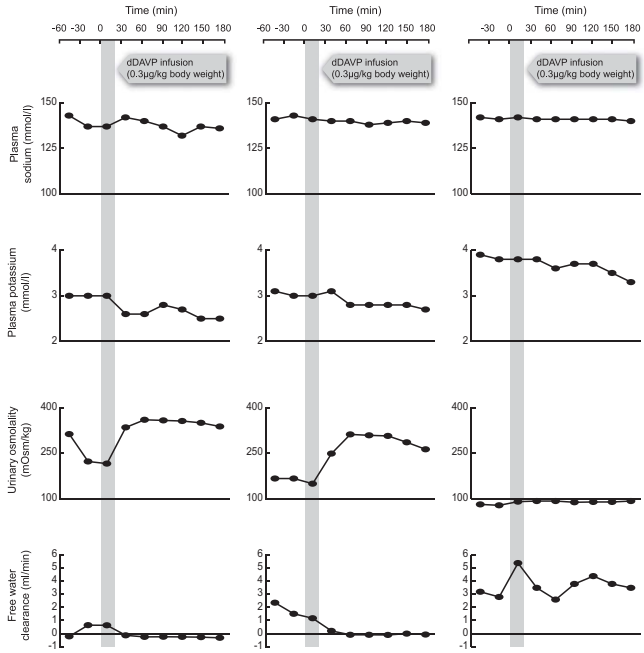

Figure 2 Measurements for 2 sisters with Bartter syndrome (left, middle columns) compared with a patient with NDI (right column) during a dDAVP infusion test. The 2 sisters with polyuria, polydipsia, hypokaliema, hypocalcemia, and nephrocalcinosis are homozygous for the A177T mutation in the *KCNJ1* gene[38] (Arthus et al, unpublished data). The dDAVP infusion tests[1] were performed at 9 (patient 1, left panel) and 11 years of age (patient 2, middle panel). Indomethacin treatment was discontinued 1 week before testing; water was not restricted during the test. The plasma vasopressin level was very low (<0.5 pg/mL) during the test, but the plasma renin activity was increased (20 ng/mL/h in patient 1, 10 ng/mL/h in patient 2). The low chloride entry in the cells of the macula densa, which is secondary to the loss of function of ROMK, explains the very high levels of plasma renin activity.[35] By contrast, patients with *AVPR2* (4-year-old patient with NDI and the *AVPR2* A132D mutation,[44] right) or *AQP2* mutations generally have low urine osmolality unresponsive to dDAVP, normal plasma potassium level, high vasopressin level, and normal plasma renin activity. Data from Fujiwara and Bichet[45] with permission.

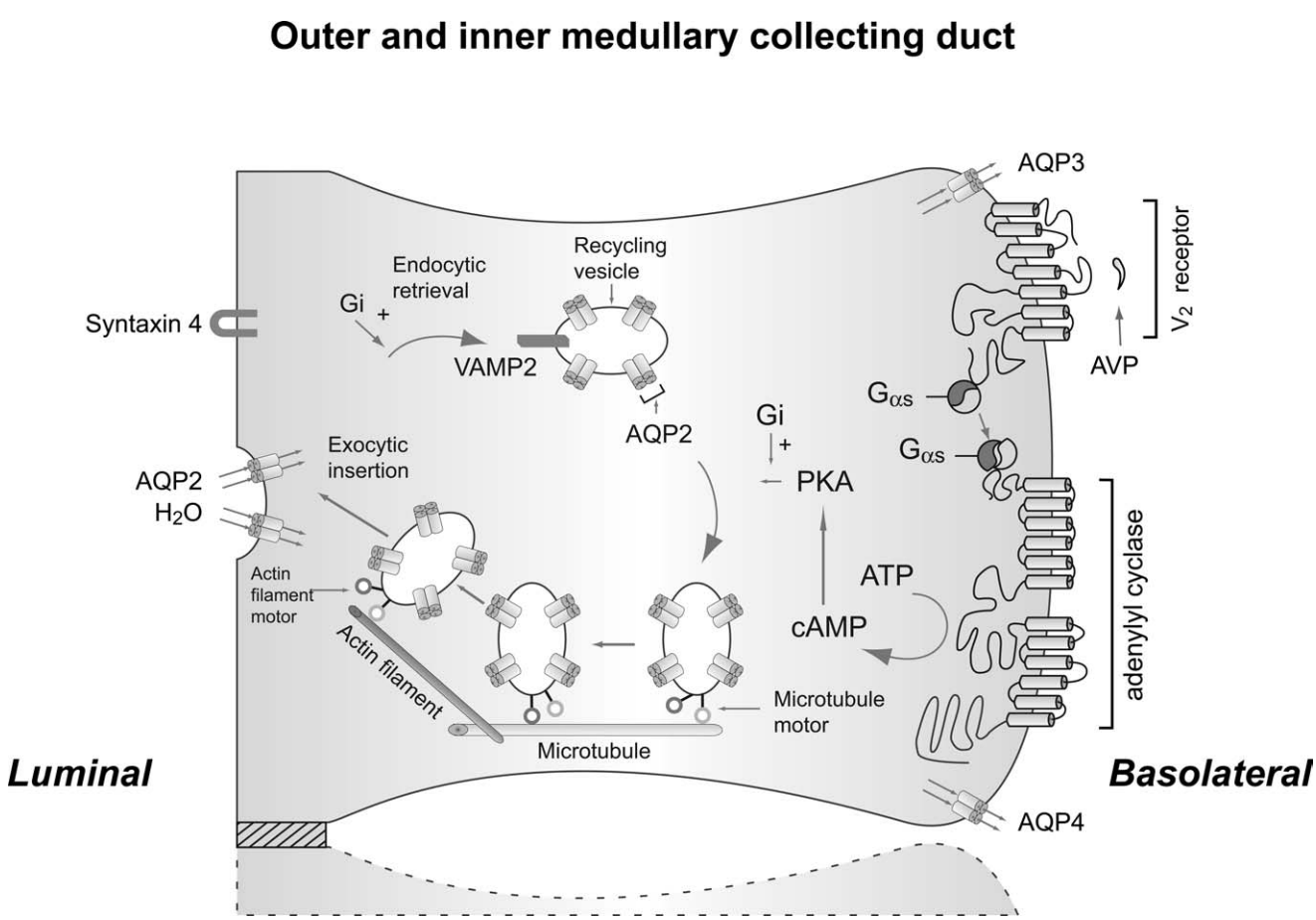

Figure 3 The effect of AVP on increasing water permeability in the principal cells of the collecting duct. Note that Na reabsorption, through the epithelial Na channel, is not represented. AVP is bound to the V_2 receptor (a G-protein–linked receptor) on the basolateral membrane. The basic process of G-protein–coupled receptor signaling consists of 3 steps: a hepta-helical receptor that detects a ligand (in this case, AVP) in the extracellular milieu, a G-protein that dissociates into α subunits bound to guanosine triphosphate and $\beta\gamma$ subunits after interaction with the ligand-bound receptor, and an effector (in this case, adenylyl cyclase) that interacts with dissociated G-protein subunits to generate small-molecule second messengers. AVP activates adenylyl cyclase, increasing the intracellular concentration of cAMP. The topology of adenylyl cyclase is characterized by 2 tandem repeats of 6 hydrophobic transmembrane domains separated by a large cytoplasmic loop and terminates in a large intracellular tail. Generation of cAMP follows receptor-linked activation of the heteromeric G-protein and interaction of the free $G_{\alpha s}$ chain with the adenylyl cyclase catalyst. 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 AVP, thereby increasing the water permeability of this membrane. Microtubules and actin filaments are necessary for vesicle movement toward the membrane. The mechanisms underlying docking and fusion of AQP2-bearing vesicles are not known. The detection of the small guanosine triphosphate binding protein Rab3a, synaptobrevin 2, and syntaxin 4 in principal cells suggests that these proteins are involved in AQP2 trafficking. When AVP is not available, water channels are retrieved by an endocytic process, and water permeability returns to its original low rate. AQP3 and AQP4 water channels are expressed on the basolateral membrane. ATP, adenosine triphosphate.

tons. In the kidney, at least 6 aquaporins are expressed: AQP1 in plasma membranes of the proximal tubule, thin descending limb of Henle, and descending vasa recta; AQP2 in the apical membrane and intracellular vesicles of collecting duct principal cells; AQP3 and AQP4 in the basolateral membranes of the same principal cells; AQP6 in intracellular vesicles of collecting duct intercalated cells; and AQP7 in the S3 segment of the proximal tubule.[3] Molecular dynamic simulations suggest that a global orientation control mechanism facilitates efficient movement of a single column of water molecules while preventing proton transport in the channel.[7] Data from mouse models suggest that AQP3 and AQP4 may play an important role in urinary concentration at the basolateral membrane.[3]

In the collecting duct, the first step in the antidiuretic action of AVP is its binding to the vasopressin V2 receptor (Fig 3) located on the basolateral membrane of collecting duct cells. This step initiates a cascade of events—receptor-linked activation of the cholera toxin-sensitive G-protein, activation of adenylyl cyclase, production of cAMP, and stimulation of protein kinase A (PKA)—which leads to the final step in the antidiuretic action of AVP. That is, the exocytic insertion of specific water channels, AQP2, into

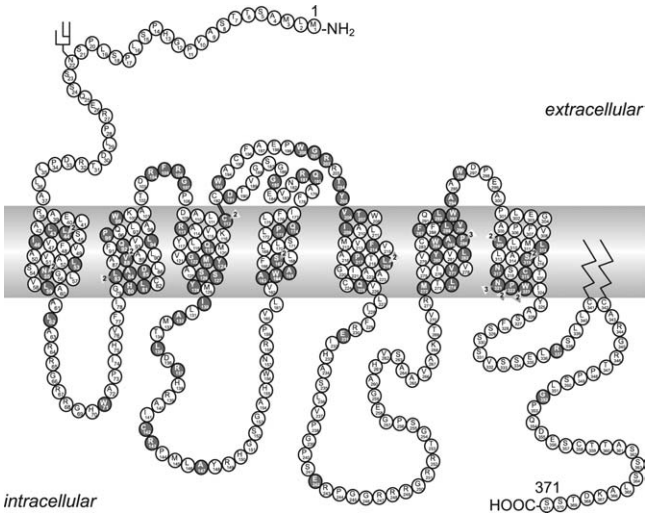

Figure 4 The V_2 receptor and identification of 183 putative disease-causing *AVPR2* mutations. Predicted amino acids are given as the 1-letter amino acid code. Solid symbols indicate missense or nonsense mutations; a number indicates more than 1 mutation in the same codon; other types of mutations are not indicated. The extracellular, transmembrane, and cytoplasmic domains are defined according to Mouillac et al.[46] There are 89 missense, 18 nonsense, 45 frameshift deletion or insertion, 7 inframe deletion or insertion, 4 splice-site, and 19 large deletion mutations, and 1 complex mutation. See http://www.medicine.mcgill.ca/nephros for a list of mutations.

the luminal membrane thereby increases the permeability of the luminal membrane. AQP2 is the vasopressin-regulated water channel in renal collecting ducts. It is present exclusively in the kidney, in principal cells of inner medullary collecting duct cells, and is distributed diffusely in the cytoplasm in the euhydrated condition, whereas apical staining of AQP2 is intensified in the dehydrated condition or after administration of dDAVP, a synthetic structural analog of AVP. The short-term AQP2 regulation by AVP involves the movement of AQP2 from intracellular vesicles to the plasma membrane, a confirmation of the shuttle hypothesis of AVP action that was proposed 2 decades ago.[8] In the long-term regulation, which requires a sustained increase of circulating AVP levels for 24 hours or longer, AVP increases the abundance of water channels. This is thought to be a consequence of increased transcription of the *AQP2* gene. The activation of PKA leads to phosphorylation of AQP2 on serine residue 256 in the cytoplasmic carboxyl terminus. This phosphorylation step is essential for the regulated movement of AQP2-containing vesicles to the plasma membrane during increases of intracellular cAMP. Phosphorylation of at least 3 of 4 monomers of an AQP2 tetramer is sufficient to redistribute AQP2 tetramers from storage vesicles to the apical membrane.[9] Drugs that disrupt microtubules or actin filaments have long been known to inhibit the hormonally induced permeability response in target epithelia[10] and Sabolic et al[11] have shown that microtubules are required for the apical polarization of AQP2 in principal cells. AQP3 and AQP4 are the constitutive water channels in the basolateral membranes of renal medullary collecting ducts.

AVP also increases the water reabsorptive capacity of the kidney by regulating the urea transporter UT-A1/3, which is present in the inner medullary collecting duct, predominantly in its terminal part.[12,13] AVP also increases the permeability of principal collecting duct cells to sodium.[14]

In summary, as stated elegantly by Ward et al,[15] in the absence of AVP stimulation, collecting duct epithelia show very low permeabilities to sodium urea and water. These specialized permeability properties permit the excretion of large volumes of hypotonic urine formed during intervals of water diuresis. In contrast, AVP stimulation of the principal cells of the collecting ducts leads to selective increases in the permeability of the apical membrane to water, urea, and Na.

X-Linked NDI (OMIM 304800): Loss-of-Function of AVPR2, Misfolding, and Functional Rescue With Nonpeptide Vasopressin Receptor Antagonists

X-linked NDI is generally a rare disease in which affected male patients do not concentrate their urine after administration of AVP.[16] Because this form is a rare and recessive X-linked disease, female individuals are unlikely to be affected, but heterozygous females can show variable degrees of polyuria and polydipsia because of skewed X chromosome inactivation. In Quebec, the incidence of this disease among male individuals was estimated to be ap-

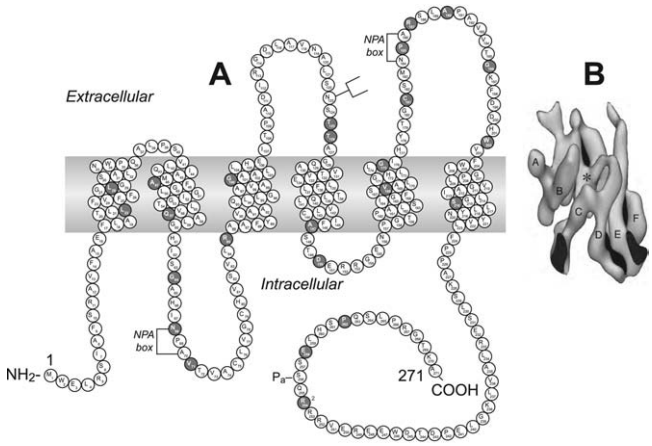

Figure 5 (A) The AQP2 and identification of 35 putative disease-causing *AQP2* mutations. See Figure 1 legend for a description of the symbols. The locations of the asparagine-proline-alanine boxes and the PKA phosphorylation site (PA) are indicated. The extracellular, transmembrane, and cytoplasmic domains are defined according to Deen et al.[47] Solid symbols indicate the location of the missense or nonsense mutations. There are 25 missense, 2 nonsense, 6 frameshift deletion or insertion, and 2 splice-site mutations. (B) A monomer is represented with 6 transmembrane helices (A-F). The asterisk indicates where the molecular pseudo-2-fold symmetry is strongest.

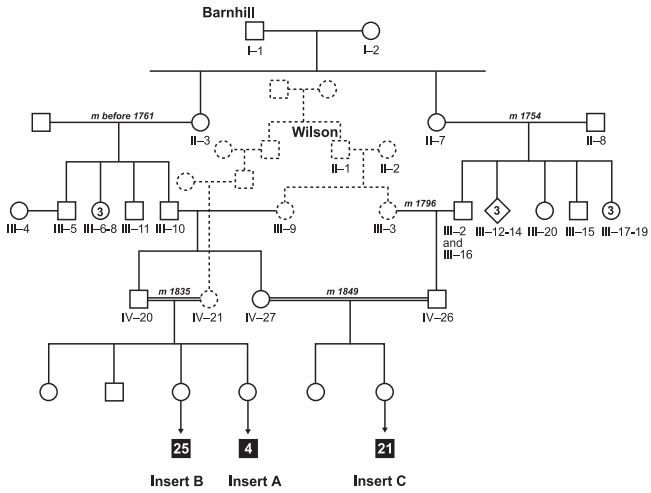

Figure 6 Follow-up of the historical reconstructions of generations I to III of the Hopewell pedigree[48] published in 1992[49] and 1993.[44] Four, 25, and 21 affected male patients now belong to inserts A, B, and C, respectively. Furthermore, our analysis of additional Maritime families confirm that carriers of the Hopewell mutation were prevalent in the Maritime area at the time the Hopewell ship landed (Arthus and Bichet, unpublished data). Therefore, the W71X mutation is not likely to be linked to Hopewell immigrants, but rather to other Ulster Scots.

proximately 8.8 in 1,000,000 male live births.[17] A founder effect of 2 particular *AVPR2* mutations, 1 in Ulster Scot immigrants (the Hopewell mutation, W71X) and 1 in a large Utah kindred (the Cannon pedigree), results in an increased prevalence of X-linked NDI in their descendants in certain communities in Nova Scotia, Canada, and in Utah (United States). These founder mutations have now spread all over the North-American continent. To date, we have identified the W71X mutation in 42 affected male individuals who reside predominantly in the Maritime Provinces of Nova Scotia and New Brunswick (Fig 6), and the L312X mutation in 8 affected male individuals who reside in the central United States. We know of 98 living affected male individuals of the Hopewell kindred and 18 living affected male individuals of the Cannon pedigree. To date, 183 putative disease-causing *AVPR2* mutations have been published in 287 NDI families (Fig 4). Eighty-nine of these 183 mutations are missense mutations likely to be misfolded, trapped in the endoplasmic reticulum and unable to reach the basolateral cell surface to engage the circulating antidiuretic hormone, AVP. Most of the *AVPR2* mutants that we and other investigators have tested are type 2 mutant receptors. They did not reach the cell membrane and were trapped in the interior of the cell.[18-21] Other mutant G-protein–coupled receptors[22] and gene products causing genetic disorders also are characterized by protein misfolding. Mutations that affect the folding of secretory proteins; integral plasma membrane proteins; or enzymes destined to the endoplasmic reticulum, Golgi complex, and lysosomes results in loss-of-function phenotypes irrespective of their direct impact on protein function because these mutant proteins are prevented from reaching their final destination.[23] Folding in the endoplasmic reticulum is the limiting step: mutant proteins that fail to fold correctly are retained initially in the endoplasmic reticulum and subsequently often degraded.

If the misfolded protein/traffic problem that is responsible for so many human genetic diseases can be overcome and the mutant protein can be transported out of the endoplasmic reticulum to its final destination, then these mutant proteins could be sufficiently functional.[24] Therefore, using pharmacologic chaperones, or *pharmacoperones*, to promote escape from the endoplasmic reticulum is a possible therapeutic approach.[23,25,26] We used selective nonpeptide V_2 and V_1 receptor antagonists to rescue the cell-surface expression and function of naturally occurring misfolded human V_2 receptors.[18] Because the beneficial effect of nonpeptide V_2 antagonists could be secondary to prevention and interference with endocytosis, we studied the R137H mutant previously reported to lead to constitutive endocytosis. We found that the antagonist did not prevent the constitutive β-arresting–promoted endocytosis.[19] These results indicate that as for other *AVPR2* mutants, the beneficial effects of the treatment result from the action of the pharmacologic chaperones. In clinical studies, we administered a nonpeptide V_{1a} vasopressin antagonist SR49059 to 5 adult patients who have NDI and bear the del62 to 64, R137H, and W164S mutations. SR49059 significantly decreased urine volume and water intake and increased urine osmolality (Fig 7), whereas sodium, potassium, and creatinine excretions and plasma sodium were constant throughout the study.[27] This new therapeutic approach could be applied to the treatment of several hereditary diseases resulting from errors in protein folding and kinesis.[24,26]

Autosomal-Recessive (OMIM 222000) and Dominant (OMIM 125800) NDI: Loss-of-Function of AQP2 (OMIM 107777), Misfolding, and/or Mistargeting

To date, 35 putative disease-causing *AQP2* mutations have been identified in 40 NDI families (Fig 5). The oocytes of the African clawed frog (*Xenopus laevis*) have provided a most useful experimental system for studying the function of many channel proteins. This convenient expression system was key to the discovery of AQP1 by Agre[28] because frog oocytes have very low permeability and survive even in fresh water ponds. Control oocytes were injected with water alone; test oocytes were injected with various quantities of synthetic transcripts from AQP1 or AQP2 DNA complementary RNA (cRNA). When subjected to a 20-mOsm osmotic shock, control oocytes had exceedingly low water permeability but test oocytes became highly permeable to water. These osmotic water permeability assays showed an absence of or very low water transport for all the cRNA with *AQP2* mutations. Immuno-

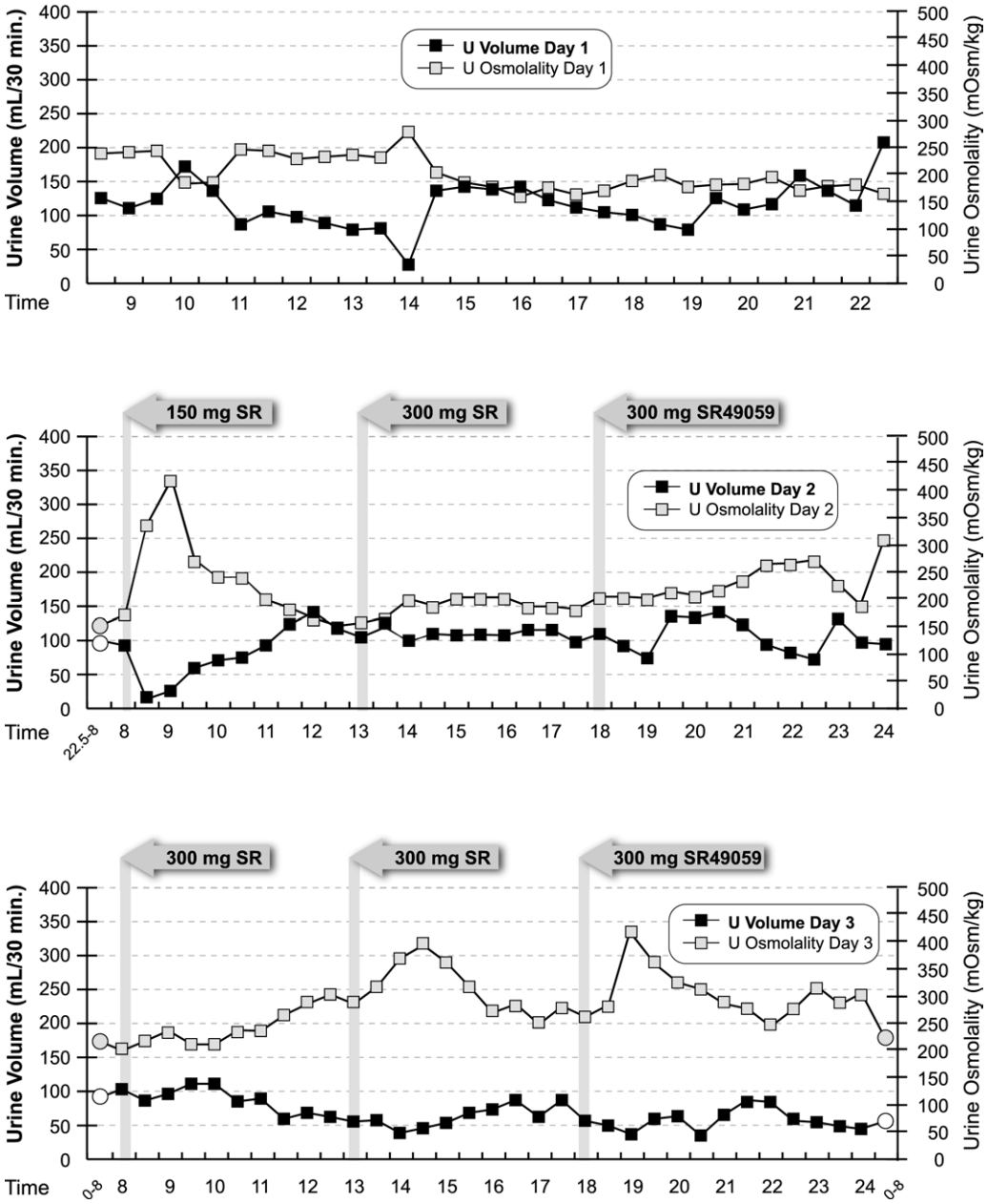

Figure 7 Urine volume and osmolality (A) before (day 1) and (B, C) after (days 2 and 3) SR49059 administration to a patient bearing the R137H mutation. (B, C) Note that the distances observed between the 2 lines on days 2 and 3 represent the mirror images of urine volume and osmolality. Urine volume and osmolalities that were obtained during the control, second, and third nights are indicated by round circles. The data were obtained from 9:30 PM to 8:00 AM for the patient described here. (A) ■, U volume day 1; □, U osmolality day 1; (B) ■, U volume day 2; □, U osmolality day 2; (C) ■, U volume day 3; □, U osmolality day 3. Data from Bernier et al[27] with permission.

fluorescence and immunoblot studies showed that these recessive mutants were retained in the endoplasmic reticulum.

AQP2 mutations in autosomal-recessive NDI, which are located throughout the gene, result in misfolded proteins that are retained in the endoplasmic reticulum. In contrast, the dominant mutations reported to date are located in the region that codes for the carboxyl terminus of AQP2.[29-31] Dominant AQP2 mutants form heterotetramers with wt-AQP2 and are misrouted. Investigation of P262L, the only recessive mutation in the carboxyl terminus, provided new insights into the loss-of-function and oligomerization of AQP2 proteins. Functional analysis in oocytes of P262L cRNA indicated that, unlike other AQP2 mutants in recessive NDI, it is a functional water channel and that trafficking to the plasma membrane was not impaired.[32] Furthermore, unlike other AQP2-recessive mutants, P262L cRNA forms heterotetramers with wt-AQP2 and is routed to the apical membrane and thus in cellular experimental systems has most of the features of AQP2 mutants in dominant NDI.

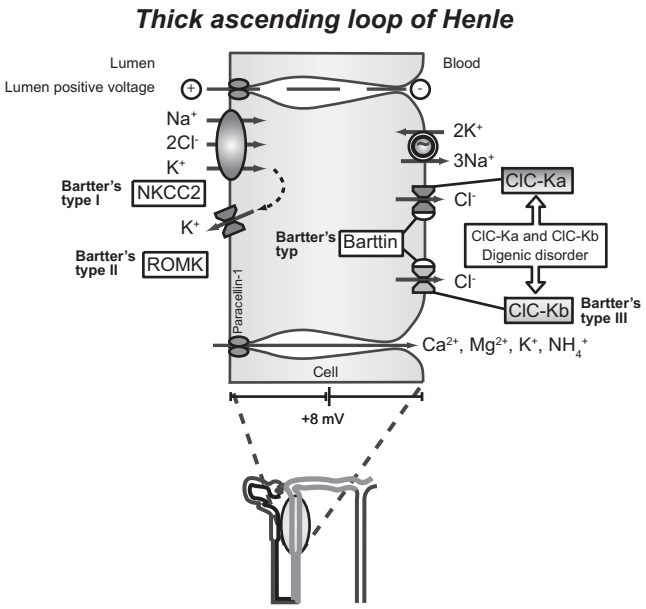

Figure 8 Transepithelial salt resorption in a cell of the thick ascending limb of the loop of Henle (TAL). Thirty percent of the filtered sodium chloride is reabsorbed in the TAL and most of the energy for concentration and dilution of the urine derives from active NaCl transport in the TAL. Filtered NaCl is reabsorbed through NKCC2, which uses the sodium gradient across the membrane to transport chloride and potassium into the cell. The potassium ions are recycled (100%) through the apical membrane by the potassium channel ROMK. Sodium leaves the cell actively through the basolateral Na-K-adenosine triphosphatase. Chloride diffuses passively through 2 basolateral channels: ClC-Ka and ClC-Kb. Both of these chloride channels must bind to the β subunit of barttin to be transported to the cell surface. Four types of Bartter syndrome (types I-IV) are attributable to recessive mutations in the genes that encode the NKCC2 cotransporter, the potassium channel (ROMK), 1 of the chloride channels (CIC-Kb), and barttin, respectively. A fifth type of Bartter syndrome also has been shown to be a digenic disorder that is attributable to loss-of-function mutations in the genes that encode the chloride channels CIC-Ka and CIC-Kb.[50] As a result of these different molecular alterations, sodium chloride is lost into the urine, positive lumen voltage is abolished, and calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), and ammonium (NH_4^+) cannot be reabsorbed in the paracellular space. In the absence of mutations, the recycling of potassium maintains a lumen-positive gradient (+8 mV). Paracellin-1 is necessary for the paracellular transport of calcium and magnesium. Modified with permission from Bichet and Fujiwara.[34]

Complex Polyuro-Polydipsic Syndrome

In contrast to a pure NDI phenotype, with loss of water but normal conservation of sodium, potassium, chloride, and calcium, in Bartter syndrome, patients' renal wasting starts prenatally and polyhydramnios often leads to prematurity. Bartter syndrome (OMIM 601678, 241200, 607364, and 602522) refers to a group of autosomal-recessive disorders caused by inactivating mutations in genes (*SLC12A1*, *KCNJ1*, *CLCNKB*, *CLCNKA*, and *CLCNKB* in combination, or *BSND*) that encode membrane proteins of the thick ascending limb of the loop of Henle (for review see Bichet[33] and Bichet and Fujiwara[34]). Although Bartter syndrome and Bartter's mutations are used commonly as a diagnosis, it is likely, as explained by Jeck et al,[35] that the 2 patients with a mild phenotype originally described by Dr. Bartter had Gitelman syndrome, a thiazide-like salt-losing tubulopathy with a defect in the distal convoluted tubule. As a consequence, salt-losing tubulopathy of the furosemide type is a more physiologically appropriate definition.

Thirty percent of the filtered sodium chloride is reabsorbed in the thick ascending limb of the loop of Henle through the apically expressed sodium-potassium-chloride cotransporter NKCC2 (encoded by the *SLC12A1* gene), which uses the sodium gradient across the membrane to transport chloride and potassium into the cell. The potassium ions must be recycled through the apical membrane by the potassium channel ROMK (encoded by the *KCNJ1* gene) (Fig 8). In the large study by Peters et al,[36] who studied 85 patients with a hypokalemic salt-losing tubulopathy, all 20 patients with *KCNJ1* mutations (except 1) and all 12 patients with *SLC12A1* mutations were born as preterm infants after severe polyhydramnios. Of note, polyhydramnios is never

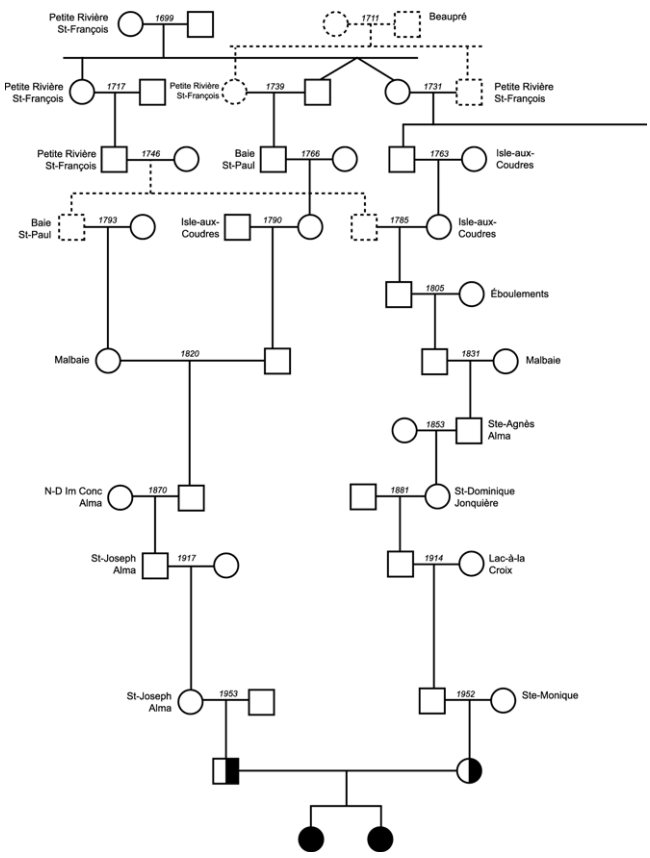

Figure 9 Reconstruction of the family tree of the 2 sisters with a complex polyuro-polydipsic syndrome secondary to loss of function of ROMK (*KCNJ1* gene). The mutation A177T likely is identical by descent from a common ancestor who lived in the Saguenay region in the 17th century. For a description of the historical, social, and genetic characteristics of the Charlevoix-Saguenay population, see Laberge et al.[51] Dates and places of marriages are indicated.

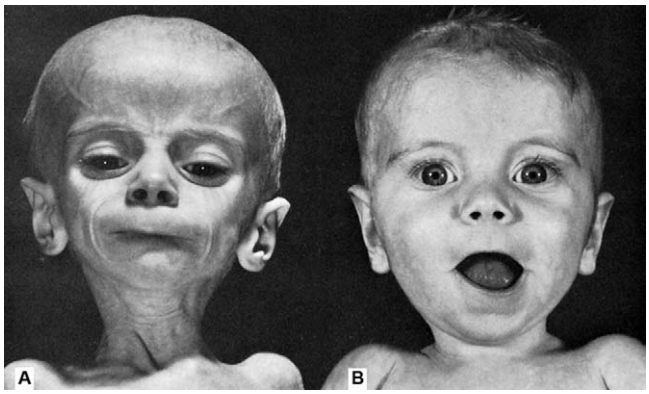

Figure 10 A typical historical picture of a dehydrated and malnourished infant (A) with NDI, and (B) looking healthy after rehydration and improved nutrition. This infant died a few years later as a result of repeated episodes of dehydration. This report was published years before the identification of the *AVPR2* gene. We were contacted by the mother and sister of this patient and we were able to reconstruct and link this family to the large Hopewell kindred[44] (Bichet and Arthus, unpublished data). The mother and sister both had the W71X mutation. Reproduced with permission from Perry et al.[52]

seen during the pregnancy that leads to infants bearing *AVPR2* or *AQP2* mutations. The most common causes of increased amniotic fluid include maternal diabetes mellitus, fetal malformations and chromosomal aberrations, twin-to-twin transfusion syndrome, rhesus incompatibility, and congenital infections.[37] Postnatally, polyuria was the leading symptom in 19 of the 32 patients. Renal ultrasound showed nephrocalcinosis in 31 of these patients. These patients, similar to the 2 young sisters described at the beginning of this presentation, with complex polyuro-polydipsic disorders often are poorly recognized and may be confused with pure NDI. As a consequence, congenital polyuria does not suggest *AVPR2* or *AQP2* mutations automatically, and polyhydramnios, salt wasting, hypokalemia, and nephrocalcinosis are important clinical and laboratory characteristics that should be assessed. In patients with Bartter syndrome (salt-losing tubulopathy/furosemide type), the dDAVP test (Fig 2) will indicate only a partial type of NDI. The algorithm proposed by Peters et al[36] is useful because most mutations in *SLC12A1* and *KCNJ1* are found in the carboxyl terminus or in the last exon and, as a consequence, are amenable to rapid DNA sequencing.

The 2 sisters with complex polyuria, hypokalemia, hypocalcemia, and nephrocalcinosis are homozygous for the A177T mutation in the *KCNJ1* gene,[38] (Arthus et al, unpublished data). Their family tree was reconstructed (Fig 9) and both parents, heterozygous for this *KCNJ1* mutation, were found to have a common ancestor from the 17th century in the Charlevoix region of Quebec. Of clinical interest, patient 2 discontinued her indomethacin treatment during a recent first pregnancy and her urine output immediately increased from 3.48 to 7.3 L/d with concomitant increases in the 24-hour urinary excretion of K^+ (57-100 mEq/d) and calcium (14.3-22.6 mEq/d). Large amounts of oral potassium supplements were necessary to maintain a plasma potassium level of 3.4 mEq/L. A normal healthy girl was delivered after an otherwise normal pregnancy.

Early Molecular Identification, Treatment, and Future Perspectives

We propose that all families with hereditary diabetes insipidus should have their molecular defect identified. This molecular identification is of immediate clinical significance because early diagnosis and treatment of affected infants can avert the physical and mental retardation that results from repeated episodes of dehydration (Fig 10). Water should be offered every 2 hours day and night, and temperature, appetite, and growth should be monitored. Admission to the hospital may be necessary for continuous gastric feeding. The voluminous amounts of water kept in patients' stomachs will exacerbate physiologic gastrointestinal reflux in infants and toddlers, and many affected boys frequently vomit. These young patients often improve with the absorption of an H_2 blocker and with metoclopramide (which could induce extrapyramidal symptoms) or with domperidone, which seems to be better tolerated and efficacious. For patients with *AVPR2* or *AQP2* mutations a low-sodium diet, distal tubule diuretics, and occasional renal prostaglandins inhibitors may achieve a 20% to 30% decrease in urine output, but the low-sodium diet is difficult to follow and affected children and adults continue to drink large amounts of water. All polyuric states (whether neurogenic, nephrogenic, or psychogenic) can induce large dilatations of the urinary tract and bladder, and bladder function impairment has been well documented in patients who bear *AVPR2* or *AQP2* mutations.[39,40] Of interest, an inducible mouse model of NDI has been produced recently by floxed *Aqp2* gene deletion,[41] which also showed evidence of structural damage from the sustained polyuria. Adult *Avpr2* or *Aqp2* knock-in mice will be useful to test new therapies including specific pharmacochaperones. Chronic renal failure secondary to bilateral hydronephrosis has been observed rarely as a long-term complication in some patients. Renal and abdominal ultrasound should be performed annually, and simple recommendations, including frequent urination and double voiding, could be important to prevent these consequences. Patients with complex polyuric disorders need to take large amounts of Na^+, K^+, and calcium and are improving dramatically with the administration of prostaglandin inhibitors.[35,42] Surviving *Romk* −/− mice[43] also will be useful to develop and test new therapies. I would like all patients with hereditary NDI to be recognized promptly, identified molecularly, and treated. The development of new pharmacologic correctors is a reachable short-term therapeutic goal.

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