Genetic forms of nephrogenic diabetes insipidus (NDI): Vasopressin receptor defect (X-linked) and aquaporin defect (autosomal recessive and dominant)

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Nephrogenic diabetes insipidus (NDI), which can be inherited or acquired, is characterized by an inability to concentrate urine despite normal or elevated plasma concentrations of the antidiuretic hormone, arginine vasopressin (AVP). Polyuria with hypo-osmolarity and polydipsia are the cardinal clinical manifestations of the disease. About 90% of patients with congenital NDI are males with X-linked NDI who have mutations in the vasopressin V2 receptor (AVPR2) gene encoding the vasopressin V2 receptor. In less than 10% of the families studied, congenital NDI has an autosomal recessive or autosomal dominant mode of inheritance with mutations in the aquaporin-2 (AQP2) gene. When studied in vitro, most AVPR2 and AQP2 mutations lead to proteins trapped in the endoplasmic reticulum and are unable to reach the plasma membrane. Prior knowledge of AVPR2 or AQP2 mutations in NDI families and perinatal mutation testing is of direct clinical value and can avert the physical and mental retardation associated with repeated episodes of dehydration.

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Cellular actions of vasopressin

The neurohypophyseal hormone arginine vasopressin (AVP) has multiple actions, including the inhibition of diuresis, contraction of smooth muscle, aggregation of platelets, stimulation of liver glycogenolysis, modulation of adrenocorticotrophic hormone release from the pituitary, and central regulation of comportemental and somatic functions (thirst avoidance, thermoregulation, increased renal sympathetic nerve activity, blood pressure) [1-4]. These multiple actions of AVP could be explained by the interaction of AVP with at least three types of G protein-coupled receptors; the V1a (vascular hepatic and various; brain cells including pre-autonomic neurons in the paraventricular nucleus responding to the dentritic release of vasopressin by neighboring magnocellular cells) and V1b (anterior pituitary) receptors act through phosphatidylinositol hydrolysis to; mobilize calcium, and the V2 (kidney) receptor is coupled to adenylate cyclase [5,6].

The transfer of water across the principal cells of the collecting ducts is now known at a very detailed level and the fact that billions of molecules of water traverse the membrane can be demonstrated as a useful teaching tool (http://www.ks.uic.edu/Gallery/Movies/aquaporin-explanation.html). The 2003 Nobel Prize in chemistry was awarded to Peter Agre and Roderick MacKinnon, who solved two complementary problems presented by the cell membrane: How does a cell let one type of ion through the lipid membrane with simultaneous exclusion of other ions? And how does it permeate water without ions? This generated great momentum and renewed interest in basic discoveries related to the transport of water and indirectly to diabetes insipidus. The first step in the action of AVP on water excretion is its binding to arginine vasopressin type 2 receptors (hereafter referred to as V2 receptors) on the basolateral membrane of the collecting duct cells (Fig. 1). The human AVPR2 gene that codes for the V2 receptor is located on chromosome region Xq28 and has three exons and two small introns [7,8]. The sequence of the cDNA predicts a polypeptide of 371 amino acids with seven transmembrane, four extracellular, and four cytoplasmic domains. The V2 receptor is one of 701 members of the rhodopsin family within the superfamily of guanine-nucleotide (G) protein-coupled receptors [9,10]. The activation of the V2 receptor on renal collecting tubules stimulates adenylyl cyclase via the stimulatory G protein (Gos2) 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 [11]. There are two target proteins for cAMP: 1) the classical protein kinase A (PKA)/cAMP-dependent protein kinase; 2) the recently discovered exchange protein directly activated by cAMP (Epac)/cAMP regulated guanine nucleotide exchange factors [12]. Like PKA, Epac contains an evolutionally conserved cAMP binding domain that acts as a molecular switch for sensing intracellular cAMP levels to control diverse biological functions. PKA and Epac may act independently on long-term regulation of AQ2P2 abundance [13].

The increase in cyclic AMP activates protein kinase A (PKA), which could phosphorylate AQ2P2 channels at five cytoplasmic carboxy-terminal tail residues, Thr 244, Ser256, Ser261, Ser264 and Ser269 (Thr269 in human AQ2P2) [14]. Ser256, Ser264 and Ser269 phosphorylations are increased in abundance upon administration of 1-desamino-8-D-arginine vasopressin (DDAVP). Phosphorylation at Ser261 is thought to stabilize AQ2P2 ubiquitination [15].

An X-ray structure of AQ2P2 at 2.75 Å resolution has been published in 2014 [16]. The structure of the carboxy- and amino-termini revealed striking differences between the X-ray structure of AQ2P2 and all other mammalian AQP structures due to the highly variable position of the short carboxy-terminal helix with high flexibility likely caused by two consecutive prolines (Pro225 and Pro226) that form a hinge region, whereas only one proline residue is present in the corresponding position in other mammalian AQP structures [16]. The crystal structure of AQ2P2 provided new structural insights to understand nephrogenic diabetes insipidus (NDI) AQ2P2 mutations affecting folding, the selectivity filter region, and the cadmium2+/calcium2+-binding sites [16].

AVP also increases the water reabsorptive capacity of the kidney by regulating the urea transporter UT-A1 that is present in the inner medullary collecting duct, predominantly in its terminal part [17]. AVP also increases the permeability of principal collecting duct cells to sodium [18].

In summary, in the absence of AVP stimulation, collecting duct epithelium exhibit 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. By contrast, AVP stimulation of the principal cells of the collecting ducts leads to selective increases in the permeability of the apical membrane to water (Pf), urea (Purea), and Na (PNa).

Four prostaglandin E2 (PGE2) receptors designated EP1–4 are expressed in the collecting duct and could control urine volume and osmolality independently of vasopressin. Olesen et al. have shown that both G-coupled PGE2 receptor EP2 and EP4 agonists increased AQP2 membrane accumulation and that the EP2 agonist butaprost relieved NDI symptoms in a rat model [19]. Moreover, a selective EP4 agonist has also been reported to be able to alleviate symptoms in a mouse model of NDI [20]. A recent study demonstrated a unique role for the EP4 receptor in controlling urine volume independently of vasopressin. EP4 activation increased collecting duct AQP2 expression in a cAMP/cAMP-response element binding protein (CREB)-dependent manner and promoted its membrane sorting via the cAMP/protein kinase A and extracellular signal-regulated kinase pathways [21].
An apical calcium/polycation receptor protein expressed in the terminal portion of the inner medullary collecting duct of the rat has been shown to reduce AVP-elicited osmotic water permeability when luminal calcium concentration rises [22]. This possible link between calcium and water metabolism may play a role in the pathogenesis of renal stone formation [22].

**Loss-of-function of AVPR2 and AQP2 are responsible for hereditary NDI**

In 1992 the AVPR2 gene encoding the V2 receptor was cloned and mutations identified in patients with X-linked NDI [23–26]. Shortly thereafter, the AQP2 gene was cloned [27,28] and in 1994 mutations in AQP2 were found to underlie autosomal; recessive Diabetes Insipidus (DI) [29]. The discovery of these 2 key genes, AVPR2 and AQP2, has allowed genetic testing of affected patients and mutations in one of them are identified in almost all patients with a clear clinical phenotype of congenital NDI [30–32].

**Rareness and diversity of AVPR2 AND AQP2 mutations**

**AVPR2 mutations**

X-linked NDI is generally a rare disease in which the affected male patients do not concentrate their urine after administration of AVP [33]. Because this form is a rare, recessive X-linked disease, females are unlikely to be affected, but heterozygous females can exhibit variable degrees of polyuria and polydipsia because of skewed X chromosome inactivation. In Quebec, the incidence of this disease among males was estimated to be approximately 8.8 in 1 000 000 male live births [31]. A founder effect of two particular AVPR2 mutations [34], one in Ulster Scot immigrants (the ‘Hopewell’ mutation, W71X) and one in a large Utah kindred (the ‘Cannon’ pedigree bearing the L312X mutation) results in an elevated prevalence of X-linked NDI in their descendants in certain communities in Nova Scotia, Canada, and in Utah, USA [34]. These founder mutations have now spread all over the North-American continent. We know of 98 living affected males of the Hopewell kindred and 20 living affected males of the Cannon pedigree. We also determined that the historical case report [35] was related to the Hopewell pedigree and had the W71X mutation (Fig. 2). To date, more than 250 putative disease-causing AVPR2 mutations have been published in more than 300 NDI families (Fig. 3) [36,37] and Bichet, unpublished data.

Approximately half of the mutations are missense mutations. Frameshift mutations owing to nucleotide deletions or insertions (25%), non-sense mutations (10%), large deletions (10%), in-frame deletions or insertions (4%), splice-site mutations, and one complex mutation account for the remainder of the mutations. Mutations have been identified in every domain, but on a per nucleotide basis, about twice as many mutations occur in transmembrane domains compared with the extracellular or intracellular domains. We previously identified private mutations, recurrent mutations, and mechanisms of mutagenesis [31,38]. The ten recurrent mutations (D85N, V88M, R113W, Y128S, R137H, S167L, R181C, R202C, A294P, and S315R) were found in 35 ancestrally independent families. The occurrence of the same mutation on different haplotypes was considered evidence for recurrence. In addition, the most frequent mutations — D85N, V88N, R113W, R137H, S167L, R181C, R202C, A294P, and S315R — occurred at potential mutational hot spots (a C-to-T or G-to-A nucleotide substitution occurred at a CpG dinucleotide).

**AQP2 mutations**

On the basis of DDAVP infusion studies and measurements of plasma cAMP levels following pharmacological intravenous doses of DDAVP, a vasopressin V2 synthetic analog, we first suggested that X-linked NDI was a pre-cAMP defect [39,40]. Male patients with X-linked NDI did not stimulate their coagulation factor release or plasma cAMP level after a pharmacological infusion of DDAVP, a suggestion of a loss of function of both renal and extrarenal vasopressin V2 receptors.

Using DDAVP infusion studies and other families with severe polyuric characteristics in both male and female individuals, a non-X-linked form of NDI with a post-receptor (post-cAMP) defect was suggested [41–43]. A patient who presented shortly after birth with typical features of NDI, but who
exhibited normal coagulation and normal fibrinolytic and vasodilatory responses to DDAVP, was shown to be a compound heterozygote for two missense mutations (R187C and S217P) in the AQP2 gene [29]. Expression of each of these two mutations in Xenopus oocytes revealed nonfunctional water channels. We used the sequencing data provided by Deen et al. to solve the molecular identification of NDI in two inbred Pakistani girls with non-X-linked NDI originally reported by Langley et al. [43]. They were found to be homozygous for the AQP2 V71M mutation. This mutation was found in two other Pakistani families living in the UK, said to be unrelated, but they were found to bear the same mutation on the same AQP2 haplotype suggesting common ancestry. To date, more than 51 putative disease-causing AQP2 mutations have been identified in 59 NDI families (Fig. 4).

Patients bearing dominant mutations have a less severe phenotype compared to patients who are compound heterozygotes or homozygotes for recessive mutations: the patient and her daughter first described to bear the AQP2 E258K dominant mutation increased their urine osmolality to 350 mOsm/kg H₂O following DDAVP [44]. Also the patient with a detailed phenotype described by Robertson and Kopp [45] increased her urine osmolality to 220 mOsm/kg H₂O during a mildly hypertonic dehydration, to 258 mOsm/kg H₂O after DDAVP and to 305 mOsm/kg H₂O after hydrochlorothiazide and indo-methacin. This patient was found to be heterozygous for the R254Q mutation, possibly interfering with the S256 phosphorylation site [46].

In the mutant Aqp2 (del 763–772) knock-in mice, Sohara et al. [47] demonstrated a slight increase in urine osmolality following dehydration but a marked increase after the administration of Rolipram, a phosphodiesterase-4 inhibitor.

In patients with hereditary polyuria, an X-linked inheritance will suggest X-linked AVPR2 mutations and autosomal recessive cases will be in favor of AQP2 mutations but we are recommending the sequencing analysis of AVPR2 first and then AQP2 genes in all patients. The coding regions of these genes are relatively small and easy to sequence. This genomic information is key to the routine care of...
patients with congenital polyuria and, as in other genetic diseases, reduces health care costs and provides psychological benefits to patients and their families.

**Benefits of genetic testing**

The natural history of untreated X-linked NDI includes hypernatremia, hyperthermia, mental retardation, and repeated episodes of dehydration in early infancy [33]. Mental retardation, a consequence of repeated episodes of dehydration (Fig. 2), was prevalent in the Crawford and Bode study, in which only nine of 82 patients (11%) had normal intelligence [48], however, data from the Nijmegen group suggest that this complication was overestimated in their group of NDI patients [49]. Early recognition and treatment of X-linked NDI with an abundant intake of water allows a normal lifespan with normal physical and mental development. Familial occurrence of males and mental retardation in untreated patients are two characteristics suggestive of X-linked NDI. Skewed X-inactivation is the most likely explanation for clinical symptoms of NDI in female carriers [31].

Identification of the molecular defect underlying X-linked NDI is of immediate clinical significance because early diagnosis and treatment of affected infants can avert the physical and mental retardation resulting from repeated episodes of dehydration. Affected males are immediately treated with abundant water intake, a low sodium diet, and hydrochlorothiazide. They do not experience severe episodes
of dehydration and their physical and mental development remains normal; however, their urinary output is only decreased by 30% and a normal growth curve is still difficult to reach during the first 2–3 years of their lives despite the aforementioned treatments and intensive attention. Water should be offered every 2 h day and night, and temperature, appetite, and growth should be monitored. Hospital admission may be necessary for continuous gastric feeding. The voluminous amounts of water kept in the patients’ stomachs will exacerbate physiological gastrointestinal reflux in infants and toddlers, and many affected boys frequently vomit and often have a gastroesophageal reflux disease (GERD) if evaluated with esophageal pH measurements (positive Tuttle test). 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.

All polyuric states (whether neurogenic, nephrogenic, or psychogenic) can induce large dilations of the urinary tract and bladder [48,50,51] and bladder function impairment has been well documented in patients carrying AVPR2 or AQP2 mutations [52,53]. Chronic renal failure secondary to

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**Fig. 4.** A representation of the AQP2 protein and identification of 46 putative disease-causing AQP2 mutations. A monomer is represented with six transmembrane helices. The extracellular, transmembrane, and cytoplasmic domains are defined according to Deen et al., 1994 [29]. Solid symbols indicate the location of the mutations: M1I; L22V; V24A; L28P; G29S; A47V; Q57P; G64R; N68S; A70D; V71M; R85X; G100X; G100V; G100R; H107D; 369delC; T125M; T126M; A147T; D150E; V168M; G175R; G180S; C181W; P185A; R187C; R187H; A190T; G196D; W202C; G215C; S216P; S216F; K228E; R254Q; R254L; E258K; and P262L. GenBank accession numbers—AQP2: AF147092, exon 1; AF147093, exons 2 through 4. NPA motifs, the N-glycosylation site, the ubiquitination site (Ub) and phosphorylation sites (P) in the C-terminus are also indicated.
bilateral hydronephrosis has been observed as a long-term complication in some of these patients. Renal and abdominal ultrasound should be done annually and simple recommendations including frequent urination and ‘double voiding’ could be important to prevent these consequences.

We propose that all families with hereditary diabetes insipidus should have their molecular defect identified. The molecular identification underlying X-linked NDI is of immediate clinical significance because early diagnosis and treatment of affected infants can avert the physical and mental retardation resulting from repeated episodes of dehydration. Diagnosis of X-linked NDI was accomplished by mutation testing of chorial villous samples \( (n = 10) \), cultured amniotic cells \( (n = 7) \), or cord blood \( (n = 57) \). Three infants who had mutational analysis done using amniotic cells or chorial villous samples also had their diagnosis confirmed by cord blood testing. Of the 74 offspring tested, 35 were found to be affected males, 22 were unaffected males, and nine were non-carriers (M.-F. Arthus, M. Lonergan, and D.G. Bichet, unpublished data). The affected males were immediately treated with abundant water intake, a low sodium diet, and hydrochlorothiazide. They have not experienced severe episodes of dehydration and their physical and mental development remains normal. Affected pre-mature males may experience less severe polyuric symptoms and may only need increased hydration during their first week without a need for hydrochlorothiazide treatment.

Most mutant V2 receptors and AQP2 water channels mutants are not transported to the cell membrane and are retained in the intracellular compartment

Misfolded AVPR2 mutants

Classification of the defects of naturally occurring mutant human V2 receptors can be based on a similar scheme to that used for the low-density lipoprotein receptor. Mutations have been grouped according to the function and subcellular localization of the mutant protein whose cDNA has been transiently transfected in a heterologous expression system [54]. Using this classification, type 1 mutant V2 receptors reach the cell surface but display impaired ligand binding and are consequently unable to induce normal cAMP production. The presence of mutant V2 receptors on the surface of transfected cells can be determined pharmacologically. By carrying out saturation binding experiments using tritiated AVP, the number of cell surface mutant V2 receptors and their apparent binding affinity can be compared with that of the wild type receptor. In addition, the presence of cell surface receptors can be assessed directly by using immunodetection strategies to visualize epitope-tagged receptors in whole-cell immunofluorescence assays.

Type 2 mutant receptors have a defective intracellular transport. This phenotype is confirmed by carrying out, in parallel, immunofluorescence experiments on cells that are intact (to demonstrate the absence of cell surface receptors) or permeabilized (to confirm the presence of intracellular receptor pools). In addition, protein expression is confirmed by western blot analysis of membrane preparations from transfected cells. It is likely that these mutant type 2 receptors accumulate in a pre-Golgi compartment, because they are initially glycosylated but fail to undergo glycosyl-trimming maturation.

Type 3 mutant receptors are ineffectively transcribed and lead to unstable mRNAs which are rapidly degraded. This subgroup seems to be rare, since Northern blot analysis of cells expressing mutant AVPR2 receptors typically show an mRNA of normal quantity and molecular size.

Most of the AVPR2 mutants that we and other investigators have tested are type 2 mutant receptors. These mutant receptors do not reach the cell membrane and are trapped in the interior of the cell [55–58]. Other mutant G-protein-coupled receptors [59] and gene products causing genetic disorders are also 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 result in loss-of-function phenotypes irrespective of their direct impact on protein function because these mutant proteins are prevented from reaching their final destination [60]. Folding in the endoplasmic reticulum is the limiting step: mutant proteins which fail to fold correctly are initially retained in the endoplasmic reticulum and subsequently often degraded. Key proteins involved in the urine countercurrent mechanisms are good examples of this basic mechanism of misfolding. AQP2 mutations responsible for autosomal recessive NDI are characterized by misrouting of the misfolded mutant proteins and are trapped in the endoplasmic reticulum [61]. This mechanism
applies to many mutated membrane proteins and, for example, mutants encoding other renal membrane proteins that are responsible for Gitelman’s syndrome [62], Bartter’s syndrome [63,64], and cystinuria [65] are also retained in the endoplasmic reticulum.

The AVPR2 missense mutations are likely to impair folding and lead to rapid degradation of the misfolded polypeptide and not to the accumulation of toxic aggregates (as is the case for AVP mutants), because the other important functions of the principal cells of the collecting duct (where the V2 receptor is expressed) are entirely normal. These cells express the epithelial sodium channel (ENaC). Decreased function of this channel results in a sodium-losing state [66]. This has not been observed in patients with AVPR2 mutations. However, recent data showed that DDAVP could not stimulate sodium reabsorption in male patients with NDI bearing AVPR2 mutations [18]. In contrast, another type of conformational disease is characterized by the toxic retention of the misfolded protein. The relatively common Z mutation in alpha-1-antitrypsin deficiency not only causes retention of the mutant protein in the endoplasmic reticulum but also affects the secondary structure by insertion of the reactive center loop of one molecule into a destabilized sheet of a second molecule [67]. These polymers clog up the endoplasmic reticulum of hepatocytes and lead to cell death and juvenile hepatitis, cirrhosis, and hepatocarcinomas in these patients [68].

Misfolded AQP2 mutants

AQP2 mutations in autosomal-recessive NDI, which are located throughout the gene, also result in misfolded proteins [16] that are retained in the endoplasmic reticulum. In contrast, the dominant mutations are located in the region that codes for the carboxyl terminus of AQP2 [69]. Dominant AQP2 mutants form heterotetramers with wild type AQP2 and are misrouted [44,46,70–78].

Nonpeptide vasopressin receptor antagonists act as pharmacological chaperones to functionally rescue misfolded mutant V2 receptors responsible for X-linked NDI

If the misfolded protein/traffic problem responsible for so many human genetic diseases can be overcome and the mutant protein transported out of the endoplasmic reticulum to its final destination, these mutant proteins could be sufficiently functional [79]. Therefore, the use of pharmacological chaperones to promote escape from the endoplasmic reticulum is a possible therapeutic approach [56,60,80]. We used selective non-peptide V2 and V1 receptor antagonists to rescue the cell surface expression and function of naturally occurring misfolded human V2 receptors [55]. Other non-peptide V2 receptor agonists were also found to have positive re-folding and functional effects [81,82]. In clinical studies, we administered a nonpeptide vasopressin antagonist SR49059 to five adult NDI patients bearing the del62-64, R137H, and W164S mutations. SR49059 significantly decreased urine volume and water intake and increased urine osmolality while sodium, potassium, and creatinine excretions and plasma sodium were constant throughout the study (Fig. 5) [83]. This new therapeutic approach could be applied to the treatment of several hereditary diseases resulting from errors in protein folding and kinesis [79,80].

Since most human gene therapy experiments using viruses to deliver and integrate DNA into host cells are potentially dangerous [84], other treatments are being actively pursued. Torsten Schöneberg and colleagues [85] used aminoglycoside antibiotics because of their ability to suppress premature termination codons [86]. They demonstrated that geneticin, a potent aminoglycoside antibiotic, increased AVP-stimulated cAMP in cultured collecting duct cells prepared from E242X mutant mice. The urine-concentrating ability of heterozygous mutant mice was also improved. The ability of EP4 to increase cellular abundance and AQP2 membrane targeting makes it a potential therapeutic target for the treatment of clinical disorders including acquired and congenital diabetes insipidus [21].

Genome editing of somatic tissue or embryos to correct mutant genes is a topic of considerable interest, but the ethics of these approaches—especially of embryo editing—are controversial [87,88] and no such treatment strategies have reached the clinic. We anticipate that the ethical governance, safety and long-term effects of editing therapies could be determined within the next two decades. Patients with hereditary disorders, including NDI, might then benefit from these curative therapies.
Over the past few years it has become clear that 'pure' (i.e. loss of water only) congenital NDI is caused by inactivating mutations in the genes that code for the V2 receptor or the AQP2 water channel [89]. The time of onset of the disease (shortly after birth) and the clinical symptoms are similar regardless of the molecular defect. The defective gene can be deduced by clinical testing: DDAVP elicits extrarenal (coagulation and vasodilatory) responses in male patients with NDI due to AQP2 mutations.

**Testing patients with NDI; please avoid dehydration**

Fig. 5. Urine volume and osmolality before (day 1) and after (days 2 and 3) SR49059 administration to a patient bearing the R137H mutation. Note that the distances observed between the two 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. These data were obtained from 9:30 p.m. to 8:00 a.m. for the patient described here. Data from Bernier [83] with permission from J Am Soc Nephrol.
whereas patients with AVPR2 mutations lack extrarenal responses [39,40,90]. Because this test is difficult to do in young infants, it has been replaced by mutational analysis of the AVPR2 and AQP2 genes. The small sizes of the genomic and coding regions of the involved genes (AVPR2 and AQP2) allow for relatively easy mutational analysis, thereby allowing for carrier, prenatal and perinatal testing. If a dehydration test or an infusion of DDAVP is done, they should be performed only during the day and under strict medical and nursing supervision.

In our clinical research unit, plasma sodium and plasma and urine osmolalities are measured at the beginning of each dehydration procedure and at regular intervals (usually hourly) thereafter, depending on the severity of the polyuric syndrome explored. In one case, an 8-year-old patient (31 kg body weight) with a clinical diagnosis of congenital NDI (later found to bear the de novo AVPR2 mutant 274insG [38] continued to excrete large volumes of urine (300 ml/h) during a short 4-h dehydration test. During this time, the patient was suffering from severe thirst, his plasma sodium was 155 mEq/l, his plasma osmolality was 310 mmol/kg, and his urine osmolality was 85 mmol/kg. The patient received 1 µg of desmopressin IV and was allowed to drink water. Repeated urine osmolality measurements demonstrated a complete urinary resistance to desmopressin. It would have been dangerous and unnecessary to prolong the dehydration further in this young patient. Thus, the usual prescription of overnight dehydration should not be used in patients, and especially children, with severe polyuria and polydipsia. Great care should be taken to avoid any severe hypertonic state, arbitrarily defined as a plasma sodium of ≥155 mEq/l.

### Practice points

- We propose that all families with hereditary diabetes insipidus should have their molecular defect identified
- The usual prescription of overnight dehydration should not be used in patients, and especially children, with severe polyuria and polydipsia. Great care should be taken to avoid any severe hypertonic state, arbitrarily defined as a plasma sodium of ≥155 mEq/l.

### Research agenda

- There is great hope to use the CRISPR (clustered regularly interspaced short palindromic repeats) gene editing technology to correct monogenic disorders but “off-target” sites are feared and it is too early to predict the human use of this promising tool in hereditary nephrogenic diabetes insipidus.
- Bypassing the V2 receptor, that is, stimulation of collecting duct cyclic AMP by other hormonal systems, is a promising experimental approach to express AQP2 channels at the luminal membrane and trials are needed to test new and re-purposed compounds to achieve this goal.

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